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VOL. XLIV JULY–AUGUST, 1952 No. 4

YEASTS ISOLATED FROM SHRIMP [1]

H. J. PHAFF,[2] E. M. MRAK [2] AND O. B. WILLIAMS [3]

The bacteriology of shrimp has received recently considerable attention (Green, **1949a**, **b**, **c**, **d**, and Holmes and McCleskey, **1949**). These studies, however, did not include information about yeasts as a part of the microflora. In fact there is very little reliable information pertaining to the occurrence of yeasts in marine products, or even in sea water. Zobell (**1946**) has pointed out that many bacteriologists have reported finding yeasts in the seas, even though special nutrient media suitable for the isolation of yeasts were not employed. Most of these reports however fail to describe the yeasts.

Fischer (**1894**) was the first to make extensive studies of the microflora of the North and South Atlantic Oceans, including the North Sea and neighboring waters. Yeasts were more numerous in coastal waters, but were regularly found at distances of several hundred miles from the nearest land and outside the common shipping lanes. However, yeasts were only found in waters located at latitudes greater than about 31°. Fischer believed that yeasts form part of the normal microflora of ocean water. The majority of the yeasts were white and red *"Torula"* species (non-fermentative, non-film forming). Near the Azores Islands a yeast was found which was described in detail in a separate publication by Fischer and

[1] Isolations were incidental to a sanitation and bacteriological study conducted by the University of Texas with financial assistance from the U. S. Public Health Service.

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Brebeck (**1894a**). It had all the characteristics of a *Sporobolomyces* species. Rarely cultures of *Mycoderma* and *Saccharomyces* were found, and then only close to land. Fischer's extensive survey showed that yeasts are widely distributed in the sea, although the identity of the organisms he studied is uncertain.

Issatchenko (**1914**) reported the general occurrence of yeasts in the Arctic Ocean, but did not identify them. Nadson and Burgwitz (**1931**) isolated from a number of species of marine algae collected in the Arctic Ocean 18 white *"Torulas,"* 7 red *"Torulas"* and one culture each of a *Dematium*-like fungus, *Oidium* and *Endomyces*. About half of the white *Torulas* could not ferment or fermented very weakly, the others were positive for a number of sugars. Of interest is the observation that some of the so-called white *Torulas* were yellow on wort agar. The "red *Torulas*" appear to be typical representatives of the now accepted genus *Rhodotorula*. All yeasts grew well at 2–4° C. The authors believed the natural habitat of these yeasts to be seaweed, since they were much more numerous on these plants than in seawater itself. Zobell and Feltham (**1934**) found yeast colonies on most plates inoculated with samples of marine materials collected near land as well as in the open ocean.

A few reports in the literature mention the occurrence of yeasts on marine products. Hunter (**1920**) found a pink yeast to be responsible for the spoilage, during shipment, of oysters grown in New England. This organism, termed *"Torula"* (probably *Rhodotorula* or *Sporobolomyces*), grew readily at low temperatures and was present in surface as well as the bottom water of oyster beds. It was also found on healthy oysters. The main source of contamination of the oysters, however, was found to be packing house equipment, which was heavily contaminated with red yeasts. Snow and Beard (**1939**) found that about 0.5 to 1.0 per cent of the organisms on fresh salmon in North Pacific waters were yeasts, but they gave no descriptions. Recently McCormack (**1950**) claimed to have observed that pink yeasts isolated from frozen oysters could grow at temperatures ranging from 0° F. to − 30° F.

During the past three years, thirty-five cultures of yeast were isolated incidental to a bacteriological study of shrimp collected off the coast of Texas. The taxonomic characteristics, salt tolerance

and temperature range for growth of these yeasts have been studied and the results are reported herein.

EXPERIMENTAL PROCEDURE

The yeasts were isolated from shrimp (*Peneaus setiferus*) collected in the Gulf of Mexico. Salinity of the water was 3.15 per cent and the temperature ranged from 28° C. to 31.8° C. Most of the yeast cultures were obtained by shaking samples of shrimp in sterile sea water (100 ml. per animal) and then culturing the washings on various types of media. All the yeast cultures except one were obtained from potato dextrose agar at pH 3.5. Culture *48–14* was isolated from dextrose tryptone agar containing 3 per

TABLE I

CULTURES ISOLATED AND SOURCES FROM WHICH THEY WERE OBTAINED

Number	Organism	Source of culture
48–10	*Torulopsis glabrata*	Surface of iced shrimp at wharf when boat unloaded at 4:00 P.M. Shrimp collected 1 mile off shore in 24 feet of water at 2:30 P.M. same day. 7/21/47.
48–11	*Trichosporon diddensii*	Surface of shrimp at time of collection 7 miles off shore in 42 feet of water. 7/15/47.
48–12	*Candida guilliermondii*	Surface of iced shrimp at wharf when boat unloaded at 6:30 P.M. Shrimp collected 4 miles off shore in 30 feet of water at 2:00 P.M. same day. 7/29/47.
48–14	*Trichosporon cutaneum*	Surface of iced shrimp at wharf when boat unloaded at 5:30 P.M. Shrimp collected 6 miles off shore in 30 feet of water at 11:30 A.M. same day. 8/30/47.
48–16	*Trichosporon cutaneum*	Surface of shrimp at packing table at about 4:00 P.M. Shrimp collected 3 miles off shore in 30 feet of water at 1:00 P.M. same day. 9/5/47.
48–17	*Trichosporon cutaneum* var. *peneaus*	Same as *48–16*
48–18	*Trichosporon cutaneum*	Surface of shrimp just after heading and washing in packing plant.
48–19	*Trichosporon cutaneum*	Same as *48–18*
48–20	*Trichosporon cutaneum*	Same as *48–18*
48–21	*Candida guilliermondii*	Surface of shrimp packed in cellophane bags and held in freezing storage from 7/17/47 to 5/29/48. Shrimp collected 2 miles off shore in 24 feet of water at 2:30 P.M. Headed, washed, packed and placed in freezing storage at 5:30 P.M., same day.

TABLE I—*Continued*

Number	Organism	Source of culture
48–22A	*Candida guilliermondii*	Same as *48–21*
48–23	*Candida parapsilosis*	Same as *48–21*, but covered with a tap water glaze.
48–23A	*Rhodotorula peneaus*	Surface washings of shrimp at the time of collection in Aransas Bay, opposite Rockport, Texas on 1/29/50.
48–23B	*Rhodotorula peneaus*	Same as *48–23A*
48–23C	*Candida parapsilosis*	Same as *48–23A*
48–23D	*Hansenula californica*	Same as *48–23A*
48–23E	*Hansenula californica*	Same as *48–23A*
48–23F	*Candida parapsilosis*	Same as *48–23A*
48–23G	*Torulopsis albida*	Same as *48–23A*
48–23H	*Trichosporon diddensii*	Same as *48–23A*
48–23I	*Trichosporon Lodderi*	Lateral plate of darkened and possibly diseased shrimp (*P. braziliensis*) at the time of unloading boat at processing plant, 9/13/49.
48–23J	*Torulopsis aeria*	Same as *48–23A*
48–23K	*Pullularia pullulans*	Same as *48–23A*
48–23L	*Pullularia pullulans*	Same as *48–23A*
48–23M	*Pullularia pullulans*	Same as *48–23A*
48–23N	*Pullularia pullulans*	Same as *48–23A*
48–23O	*Rhodotorula texensis*	Same as *48–23I*
48–23P	*Rhodotorula marina*	Same as *48–23A*
48–23Q	*Rhodotorula glutinis*	Same as *48–23A*
48–23R	*Rhodotorula glutinis*	Same as *48–23A*
48–23S	*Rhodotorula mucilaginosa*	Same as *48–23A*
48–23T	*Rhodotorula glutinis*	Same as *48–23A*
48–23U	*Rhodotorula* (*48–23O*) *texensis*	Same as *48–23A*
48–23V	*Rhodotorula mucilaginosa*	Same as *48–23A*
48–23W	*Rhodotorula glutinis*	Same as *48–23A*

cent NaCl and at pH 6.8. The plates were incubated at room temperature (about 30° C.) for 5 days and colonies were picked to agar slants for later study. Culturings were made at various steps in the shrimp-handling operation. (See TABLE I.) Culture *48–23J* was obtained from intestinal contents of shrimp, and *48–23I* from the lateral plate of a darkened and possibly diseased shrimp.

Taxonomic procedures used were similar to those of Lodder (**1934**), Diddens and Lodder (**1942**) and Wickerham (**1951**). In addition the carotenoid test of Mrak *et al.* (**1950**), sporulation medium of Mrak, Phaff and Douglas (**1942**), enriched with 1 per cent dried Fleischmann yeast type 20–40, and Wickerham's (**1951**) carbon and nitrogen base (Difco) (for the preparation of auxanogram media and alcohol medium), were also employed. Starch production was tested by the procedure of Mager and Aschner

(**1946, 1947**). Thallus characteristics were observed in moist chamber slides as well as by the customary pseudomycelium procedure and in some cases as Dalmau preparations (see Wickerham, **1951**).

Salt tolerance tests were made with 10 per cent wort agar slants containing NaCl ranging in concentration from 8 to 16 per cent. Temperature tolerance for growth was determined by inoculation of 10° Balling wort agar slants from young liquid wort cultures and incubation at various temperatures ranging from 0 to 46° C. The period of incubation ranged from 3–7 days at temperatures above 15° C. (depending on the amount of growth) and up to 3 weeks at the lower temperatures.

EXPERIMENTAL OBSERVATIONS

Thirty-five yeast cultures were isolated from shrimp (*Penaeus setiferus*) collected off the coast of Texas in the Gulf of Mexico (see TABLE I). The isolates were found to belong to the genera *Rhodotorula, Trichosporon, Torulopsis, Pullularia, Candida,* and *Hansenula.* Their taxonomic position is as follows:

Genus *Rhodotorula:* None of the cultures of *Rhodotorula* corresponded exactly with described species; however, in a number of cases the differences were minor. Four cultures were designated as *R. glutinis* (*48–23Q, 48–23R, 48–23T, 48–23W*). These isolates fit well in Lodder's group consisting of *R. glutinis* and its four varieties. In the opinion of the authors it is very difficult to separate isolates falling in this group into varieties on the basis of the relatively insignificant characteristics (such as color intensity and minor differences in shade and cell size) which were used by Lodder, although the senior author has recently shown (unpublished results) that there exist certain distinct biochemical differences in carbon assimilation reactions between the authentic strains of the *Rhodotorula glutinis* group as well as the strains from shrimp. Nevertheless the latter will be classified in the *glutinis* group until this study of the genus *Rhodotorula* is completed.

The four cultures also differed in vitamin requirements, as evidenced by the growth in ethyl alcohol medium without vitamins, and that in the presence of vitamins. The addition of vitamins improved the growth and changed the color of strains *48–23T* and

48–23W in ethanol from fair with whitish cells to heavy with slimy film and bright red cells. The other two strains were not stimulated.

Two cultures were designated as *R. mucilaginosa* (*48–23S* and *48–23V*). They fit well in the group comprising *R. mucilaginosa* and its four varieties as defined by Lodder. However, as in the case of *R. glutinis*, the two isolates differed from each other and the type species and varieties by a number of variations in carbon assimilation. Culture *48–23P* could not be identified with any of the known species of *Rhodotorula*. It resembles *R. rubra*, but differs in that it utilizes lactose (after a short adaptation period), it fails to utilize alcohol even in the presence of added growth factors, and in slant culture characteristics. Furthermore there are appreciable differences between this isolate and the three strains described by Lodder in assimilation of certain carbon compounds not used by her. These differences are sufficient to consider this isolate a new species. We propose the name *Rhodotorula marina*, since this organism is of marine origin and may have its normal habitat in the sea.

Rhodotorula marina sp. nov.:

Post dies tres in temperatura vulgari crescunt in extracto maltoso cellulae ellipticae vel globosae vel elongatae, singulae vel binae vel trinae vel quaternae, 2.6–5.1 × 3.1–11.5 μ. Conspicuus anulus formatur sed non pellicula. Linea valide rosea, levis, corusca. Textus glutinosus sed non mucosus, convexus cum margine integro. Fermentatio non occurrit. Et glucosum, et galactosum (exigue); et sucrosum, et maltosum, et lactosum (post adaptationem) assimilantur. Nitrati non utitur. In urea, et sulfate ammonii et asparagino exigue crescit; in peptono bene crescit. Ethanolo non utitur praesentibus vitaminis. Arbutinum non finditur. Amylum non componitur. Acidus non formatur in agaro cretaceo. Varietas temperaturae ad crescendum tenuis, 32° C. non crescit et 10° C. vix crescit.

Standard description of *Rhodotorula marina*:

Growth in malt extract: After 3 days at room temperature cells ellipsoidal, globose or elongate. They occur singly or in pairs or in small clusters of 3 or 4 cells. Cell size, 2.6–5.1 × 3.1–11.5 μ. A distinct ring is formed, but no pellicle.

Growth on wort agar: Streak deep pink, smooth, glistening. Texture pasty but not slimy, convex with entire border. No fermentation. Assimilates glucose, galactose (weak), sucrose, mal-

tose and lactose (after adaptation). Nitrate not utilized. Urea, ammonium sulfate and asparagine give weak growth and peptone good growth. Ethanol not utilized in presence of vitamins. Arbutin not split. Starch not synthesized. No acid produced on chalk containing agar. Temperature range of growth narrow; none at 32° C. and poor at 10° C.

Cultures *48–23O* and *48–23U* were similar and could not be identified with any known *Rhodotorula* species. The sugar assimilation pattern (maltose − and lactose +) and the absence of nitrate utilization make it impossible to fit them in any described species. Since it has only been found in Texas, we suggest the name *Rhodotorula texensis*. Culture *48–23U* has been chosen as the type organism.

Rhodotorula texensis sp. nov.:

Cellulae globosae vel ellipticae, binae vel trinae vel quatternae sed non pseudomycelium, 2.5–5 × 3–6 μ, in herbacea cultura iuvenili, cellulae progemmantes in lata basi vel undique. Cellulae pigmenta carotenoida habent quae in cultura praebent speciem roseam. In herbacea cultura liquida conspicuus anulus et sedimentum formantur sed non pellicula. Fermantatio non occurrit. Glucosum, et galactosum, et sucrosum, et lactosum assimilantur sed non maltosum. Asparagino, et urea et $(NH_4)_2SO_4$ et peptono utitur sed non KNO_3. In medio alcohol sine auxiliis crescendi non bene crescit sed in medio Wickerhami cum alcohol mediocriter. Nulla productio acidi in medio Gusteri. Lac coagulatur, pinguedo non finditur. Amylum non componitur rebus faventibus. Arbutinum exiguissime finditur. Cultura rosea, levis, corusca; textus glutinosus non mucosus; forma convexa; margo integer.

Standard description of *R. texensis:*

In young wort cultures: Cells globose or ellipsoidal, in pairs or groups of 3 or 4 but no pseudomycelium, 2.5–5 × 3–6 μ, budding on a broad base or on all sides. Cells contain carotenoid pigments giving the culture a pink appearance. In liquid wort culture a distinct ring and sediment but no film. No fermentation. Assimilation of glucose, galactose, sucrose and lactose, but not of maltose. Asparagin, urea, $(NH_4)_2SO_4$ and peptone utilized but not KNO_3. Poor growth in alcohol medium without growth factors, but fair in Wickerham's medium plus alcohol. No acid formation on Custer's medium. Milk coagulated. No fat splitting. Starch not synthesized under appropriate conditions. Arbutin test very weakly positive. Slant culture pink, smooth, glistening, texture not slimy but paste like, form convex and border entire.

Cultures *48–23A* and *48–23B* were different from any of the known species of *Rhodotorula*. According to Lodder's key they resemble *R. aurea* or *R. flava*. However, our cultures differ from these species by their inability to assimilate ethanol with added growth factors, a smaller width of the cells and the brighter, more canary yellow appearance of the streak. Although these differences do not appear great, there exist numerous biochemical differences in carbon assimilation tests, which will not be considered in the present publication, but which induced us to describe these organisms as a new species. The isolates may be similar to the yellow yeasts mentioned by Nadson and Burgwitz (**1931**). Since they were isolated from shrimp of the genus *Peneaus* we propose the name *Rhodotorula peneaus*. Culture *48–23A* was chosen as the type.

Rhodotorula peneaus sp. nov.:

Cellulae globosae vel ellipticae, singulae vel binae, 2–3.5 × 2.6–7 μ in cultura iuvenili in liquida herbacea; progemmantes in basi lata vel undique. Cellulae, habentes carotenoidea pigmenta, producunt species valide flavidas. In liquida herbacea anulus flavus et sedimentum formantur, sed non pellicula. Aliquando paucae et leves insulae. Fermentatio non effecta. Assimilatio glucosi, galactosi, sucrosi, maltosi lactosique, $(NH_4)_2SO_4$, urea, asparagino, et peptono utitur sed non KNO_3. In medio alcoholicus, cum auxiliis crescendi, minime crescit. Nulla productio acidi in agaro Custeri. Lac non coagulatur, pinguedo non finditur. Amylum exigue componitur. Arbutinum finditur. Cultura flavida vel valide flava, levis, corusca, mollis, mucosa (fluit); forma convexa; margo integra.

Standard description of *R. peneaus:*

Cells globose to ellipsoidal, single or in pairs, 2–3.5 × 2.6–7 μ in young liquid wort cultures. Budding on a broad basis or on all sides. Cells contain carotenoid pigments giving the culture a canary yellow appearance. In liquid wort culture a yellow ring and sediment but no film. Occasionally a few shiny islands. No fermentation. Assimilation of glucose, galactose, sucrose, maltose and lactose, $(NH_4)_2SO_4$, urea, asparagine and peptone are utilized, but not KNO_3. In alcohol medium containing growth factors practically no growth. No acid formation on Custer's agar. No coagulation of milk, or splitting of fat. Starch synthesis weakly positive. Arbutin splitting positive. Slant culture yellow to canary yellow, smooth, glistening, soft and slimy (runs), form convex and border entire.

The genus *Trichosporon:* Nine cultures belonged to this genus. Five were designated as *Tr. cutaneum* (*48–14, 48–16, 48–18, 48–19* and *48–20*). They varied from the description of Diddens and Lodder by forming a thin pellicle and showing better growth in alcohol medium without added growth factors. If vitamins were added to this medium growth became heavy and the film was thicker. These organisms were also able to split fat weakly. They grew well in milk, but showed no coagulation, whereas Diddens and Lodder reported this to be positive. Very little acid was produced in milk and likewise there was no evidence of acid formation on Custers' agar. These differences, however, are of a minor nature. One isolate (*48–17*) differed from *Tr. cutaneum* in its sugar assimilation reactions. The type species assimilates glucose, galactose, sucrose, maltose and lactose on an auxanogram plate whereas *48–17* showed a negative reaction with sucrose. However, in Wickerham's liquid assimilation medium a delayed sucrose utilization occurred and after 20 days a strongly positive growth could be observed. Furthermore it differed from the other five isolates of *Tr. cutaneum* by having a much higher maximum growth temperature (see TABLE III) and by showing a weak synthesis of a starch-like compound under appropriate conditions. Since Diddens and Lodder established the variety *Tr. cutaneum* var. *infestans* on the basis of a different sugar assimilation pattern, we have decided to do likewise and propose the name *Trichosporon cutaneum* var. *peneaus* for our isolate.

TRICHOSPORON CUTANEUM var. **peneaus** var. nov.:

In et liquida et iuvenili herbacea cultura pellicula hebes, rugosa et densa. Mycelium et arthrosporae maxime abundantes. Blastosporae paucae. Rapida assimilatio glucosi et galactosi et maltosi et lactosi. Sucroso utitur tarde. In medio alcohol, praesentibus vitaminis, abundanter crescit pellicula hebes, levis, facile decidua. Arbutinum non finditur. Rebus faventibus compositum amylosum exique componitur.

Supplementary description of *Tr. cutaneum* var. *peneaus:*

In young liquid wort cultures dull, rugose, thick film. Mycelium and arthrospores most abundant. Blastospores few. Rapid assimilation of glucose, galactose, maltose and lactose. Sucrose

utilized slowly. In alcohol medium with vitamins heavy growth with a smooth dull film, which falls easily. Arbutin not split. Weak synthesis of a starch-like compound under appropriate conditions.

The remaining 3 isolates were fermentative species of *Trichosporon*. Diddens and Lodder have described only one fermentative species, *Tr. fermentans*. Cultures *48–11* and *48–23H* have the same fermentation characteristics as *Tr. fermentans* (glucose and galactose), but they differ from *Tr. fermentans* by assimilating sucrose and maltose.

In honor of the late Dr. Diddens we propose to name these isolates *Trichosporon diddensii*. Culture *48–23H* has been chosen as the type.

Trichosporon diddensii sp. nov.:

Tertia die in temperatura vulgari crescunt in extracto maltoso cellulae globosae vel longae et cylindricae, 2.3–4.4 × 3.1–7.0 μ. In cultura iuvenili typus globosus superat. Cellulae hyphales sunt 1.7–2.5 × 5–40 μ. In thallo cellulae sunt binae vel aggregatae, pseudomycelium habentes, in vetustioribus herbaceis culturis verum mycelium et arthrosporas. Grave sedimentum, anulus facile deciduus formantur. Pellicula deest. Cultura lineata, in herbaceo arago, albida, superficie circumferentiae nitida, corusca, in parte media leviter rugosa, semi-corusca. Textus glutinatus; emargine integro diffuse excrescit pseudomycelium. Pseudomycelium aliquantum primitivum et non extensum in cultura agari dextrosi solanacei. Saepe crescit desuper in agarum. Arthrosporae videntur in iure humido. Fermentatio glucosi et galactosi exigua. Nulla fermentatio maltosi, sucrosi, raffinosi, lactosique. Glucosum, et galactosum, et sucrosum et maltosum assimlantur sed non lactosum. KNO_3 non utitur. Urea et sulfate ammonii leviter et asparagino et peptono praesentibus vitaminis fortiter utitur. Tenuis anulus et mediocre sedimentum in medio alcohol ethyl praesentibus vitaminis. Sine vitaminis solum sedimentum; mediocriter vel male crescit. Arbutinum finditur. Amylum non componitur. Acidus non formatur. Pinguedo non finditur. Lac non coagulatur.

Standard description of *Trichosporon diddensii:*

Growth in malt extract: Three days at room temperature, cells globose to long cylindrical. The globose type predominates in young cultures and is 2.3–4.4 × 3.1–7 μ. The hyphal cells are 1.7–2.5 × 5–40 μ. Thallus consists of pairs, clusters and pseudomycelium and in older wort cultures true mycelium and arthrospores. Ring is formed, which falls easily. No film. Heavy sediment. Streak culture on wort agar white. Surface of periph-

ery smooth and glistening, center finely rugose, semi-glistening. Texture pasty, growth rather spreading. Border entire with pseudomycelial outgrowth. Pseudomycelium rather primitive and not extensive on potato dextrose agar slide culture. Tendency to grow down into agar. Arthrospores apparent in wet mount. Fermentation of glucose and galactose (weak). Maltose, sucrose, raffinose and lactose not fermented. Assimilation of glucose, galactose, sucrose and maltose. Lactose not assimilated. KNO_3 not utilized. Urea and ammonium sulfate utilized weakly and asparagin and peptone strongly in the presence of vitamins. Thin ring and moderate sediment in ethyl alcohol medium (with vitamins). Without vitamins only sediment; moderate to poor growth. Arbutin split. Starch not synthesized. No acid formation. No fat splitting. No coagulation of milk.

The third isolate (*48–231*) fermented, in addition to glucose and galactose, sucrose and maltose, but did not ferment one-third of the raffinose molecule. These characteristics are sufficient to justify naming this organism as a new species and we propose the name *Trichosporon lodderi*.

Trichosporon lodderi sp. nov.:

Post tres dies in temperatura vulgari crescunt in extracto maltoso cellulae globosae vel elongatae vel cylindricae; haec faciunt pseudomycelium. Cellulae globosae et elongatae plerumque vel singulae vel binae 3.3–7.0 × 3.8–13.0 μ. Multiplex formatio anulorum sine pellicula. Post diem 30 mycelium verum occurrit. Cultura lineata in herbaceo agaro, albida, plicata; hebes pulvinosa; textus cum margine piloso. In cultura in solanaceo dextroso agaro bene crescunt mycelium verum, pseudomycelium, blastosporae, blastoconidia et arthrosporae. Fermentatio glucosi, galactosi (exiguis); maltosae, sucrosi (mediocris). Fermentatio et raffinosi et lactosi non occurrit. Glucosum, et galactosum, et sucrosum et maltosum assimilantur, sed non lactosum. KNO_3 non utitur. Ureae sulfate et ammonia et asparagino et peptono bene utitur praesentibus vitaminis. In medio ethyl alcohol cum vitaminis, densus anulus et grave sedimentum formantur. Sine vitaminis male crescit. Arbutinum non finditur. Amylum non componitur. Acidus non formatur. Pinguedo non finditur. Lac non coagulatur.

Standard description of *Trichosporon lodderi:*

Growth in malt extract after three days at room temperature: Cells globose, elongate or cylindrical, the latter forming pseudomycelium. Globose and elongate cells usually occur singly or in pairs and are 3.3–7 × 3.8–13 μ. Repeated ring formation but no pellicle. After 30 days true mycelium present. Streak culture on

wort agar white, corrugated, dull powdery, texture tough, border hairy. On potato dextrose agar slide culture a well developed true mycelium and pseudomycelium, blastospores, blastoconidia and arthrospores. Fermentation of glucose, galactose (weak), maltose and sucrose (fair). Raffinose and lactose not fermented. Assimilation of glucose, galactose, sucrose and maltose but not of lactose. KNO_3 not utilized. Urea, ammonium sulfate, asparagine and peptone utilized well in the presence of vitamins. Ethyl alcohol medium (with vitamins) thick ring, heavy sediment. Without vitamins poor growth. Arbutin not split. Starch not synthesized. No acid formation. No fat splitting. No coagulation of milk.

The genus *Candida:* Six isolates were found to belong to *Candida.* Three isolates (*48–12, 48–21* and *48–22A*) were considered similar to *C. guilliermondii.* They checked quite well with the description given by Diddens and Lodder and belong among those strains characterized by a primitive pseudomycelium. The other three isolates (*48–23, 48–23C* and *48–23F*) were identified as *C. parapsilosis.* Growth in alcohol medium of the latter three cultures was strongly stimulated by the addition of vitamins, and culture *48–23C* formed under those conditions, in addition, a moist, slimy, fairly thick, but fragile film; whereas *48–23* and *48–23F* only formed a ring and heavy sediment.

The genus *Torulopsis:* Two isolates belonged to this genus. Culture *48–23J* was similar to *T. acria* and culture *48–10* was similar to *T. glabrata.*

The four cultures of black yeast were considered to be *Pullularia pullulans,* although they were not studied as intensively as the others.

The remaining two isolated (*48–23D* and *48–23E*) were similar and were the only two sporulating cultures found. They belong to the genus *Hansenula,* because of the ability to show strong growth with nitrate as the single source of nitrogen. At first ascospores could not be found in spite of the fact that on several sporulation media numerous empty dumbbells were found, distinct evidence of conjugation. Later it was observed that the conjugating cells were swollen ascospores and that the most distinct phase of the life cycle consisted of the fusion dumbbells of two ascospores, restoring the diploid phase. Later ascospores were seen in small numbers in

young cultures. They are spheroid, usually two per ascus and of very short duration. Soon after their formation they swell, the ascus ruptures and disintegrates and the spores look very much like vegetative cells. Conjugation usually follows directly after their germination. Following Wickerham's (**1951**) new key for *Hansenula* these organisms fit *H. californica,* although distinct saturn-shaped spores were not seen and esters were not produced.

SALT TOLERANCE

Most of the yeasts were isolated from shrimp shortly after removal from water containing 3.15 per cent salt. An experiment

TABLE II

GROWTH ON 8.5° BALLING WORT AGAR CONTAINING VARIOUS AMOUNTS OF SALT

Code	Culture	Growth at various concentrations of salt (in per cent by weight)					
		8.3[a]	9.8	12	13.4	14.7	16
48–23O	*Rhodotorula glutinis*	+++[b]	+++	+++	++	++	±
48–23R	*Rhodotorula glutinis*	+++	+++	+++	++	++	±
48–23T	*Rhodotorula glutinis*	+++	+++	+++	++	++	+
48–23W	*Rhodotorula glutinis*	+++	+++	+++	+++	+++	+++
48–23S	*R. mucilaginosa*	+++	+++	++	++	++	±
48–23V	*R. mucilaginosa*	+++	+++	++	++	++	±
48–23P	*R. marina*	+	±	−	−	−	−
48–23A	*R. peneaus*	+++	+++	+++	+++	++	±
48–23B	*R. peneaus*	+++	+++	+++	+++	++	±
48–23G	*Torulopsis albida*	+++	++	−	−	−	−
48–23O	*R. texensis*	++	+	−	−	−	−
48–23U	*R. texensis*	+++	+	−	−	−	−
48–14	*Trichosporon cutaneum*	−	−	−	−	−	−
48–16	*Trichosporon cutaneum*	±	−	−	−	−	−
48–18	*Trichosporon cutaneum*	++	+	−	−	−	−
48–19	*Trichosporon cutaneum*	++	+	−	−	−	−
48–20	*Trichosporon cutaneum*	++	+	−	−	−	−
48–17	*Trichosporon cutaneum* var. *peneaus*	++	+	−	−	−	−
48–23H	*Trichosporon diddensii*	+++	+++	+++	+++	++	++
48–11	*Trichosporon diddensii*	+++	+++	+++	+++	++	±
48–23I	*Trichosporon lodderi*	+++	+++	+++	++	++	−
48–12	*Candida guilliermondii*	+++	+++	+++	+++	++	+
48–21	*Candida guilliermondii*	+++	+++	+++	+++	++	+
48–22A	*Candida guilliermondii*	+++	+++	+++	+++	++	+
48–23	*Candida parapsilosis*	+++	+++	+++	++	++	++
48–23J	*Torulopsis aeria*	+++	++	−	−	−	−
48–10	*Torulopsis glabrata*	+	−	−	−	−	−
48–23D	*Hansenula californica*	±	−	−	−	−	−
48–23E	*Hansenula californica*	±	−	−	−	−	−
48–23C	*Candida parapsilosis*	+++	+++	+++	+++	+	+
48–23F	*Candida parapsilosis*	+++	+++	+++	+++	++	+

[a] Observations made after 7 days on slants containing 8.3–13.4 per cent salt; 12 days for those with 14.7 and 16 per cent salt.

[b] ± = questionable growth.
+ = little growth.
++ = moderate growth.
+++ = heavy growth.

TABLE III

GROWTH AT VARIOUS TEMPERATURES*

Code	Culture	Temperature in degrees C.								
		0	2.5	5	10	14	19	32	38	41.5
48–23Q	*R. glutinis*	±	++	++++	++++	++++	++++	+++	−	−
48–23R	*R. glutinis*	−	++	++++	++++	++++	++++	+++	−	−
48–23T	*R. glutinis*	−	±	++++	++++	++++	++++	+++	−	−
48–23W	*R. glutinis*	−	−	++++	++++	++++	++++	+++	−	−
48–23S	*R. mucilaginosa*	−	+	+++	++++	++++	++++	+++	−	−
48–23V	*R. mucilaginosa*	−	−	+++	++++	++++	++++	+++	−	−
48–23P	*Rhodotorula marina*	−	−	−	+	++	++	−	−	−
48–23A	*Rhodotorula peneaus*	−	+	++++	++++	++++	++++	+	−	−
48–23B	*Rhodotorula peneaus*	−	++	++++	++++	++++	++++	++	−	−
48–23G	*Torulopsis albida*	−	±	+++	++++	++++	++++	++++	−	−
48–23O	*R. texensis*	−	−	+++	++++	++++	++++	+++	+	−
48–23U	*R. texensis*	−	−	+++	++++	++++	++++	+++	−	−
48–14	*Trichosporon cutaneum*	−	−	−	+++	++++	++++	++++	−	−
48–16	*Trichosporon cutaneum*	−	−	−	+	+++	++++	++++	−	−

* The results at 0, 2.5, 5° C. are recorded after 23 days of growth; those at 10° after 13 days; at 14° C after 8 days; and at the remaining temperatures after 3 days.

None of the cultures grew at 46° C.

TABLE III—*Continued*

Code	Culture	Temperature in degrees C.								
		0	2.5	5	10	14	19	32	38	41.5
48–18	*Trichosporon cutaneum*	−	−	±	++++	++++	++++	++++	±	−
48–19	*Trichosporon cutaneum*	−	−	+	++++	++++	++++	++++	±	−
48–20	*Trichosporon cutaneum*	−	−	−	++++	++++	++++	++++	±	−
48–17	*Trichosporon cutaneum* var. *peneaus*	−	−	−	±	++++	++++	++++	++++	+++
48–23H	*Trichosporon diddensii*	−	−	−	++++	++++	++++	+++	++	−
48–11	*Trichosporon diddensii*	−	−	−	++++	++++	++++	++++	±	−
48–23I	*Trichosporon lodderi*	−	−	−	++	++++	++++	++++	+++	−
48–12	*Candida guilliermondii*	−	±	++++	++++	++++	++++	++++	+++	−
48–21	*Candida guilliermondii*	±	+	++++	++++	++++	++++	++++	+++	−
48–22A	*Candida guilliermondii*	−	±	++++	++++	++++	++++	++++	+++	−
48–23	*Candida parapsilosis*	−	−	+	++++	++++	++++	++++	++	−
48–23J	*Torulopsis aeria*	−	++	++++	++++	++++	++++	++++	−	−
48–10	*Torulopsis glabrata*	−	−	++	+++	++++	++++	+++	+++	+++
48–23D	*Hansenula californica*	−	−	+++	++++	++++	++++	+++	−	−
48–23E	*Hansenula californica*	−	−	+++	+++	++++	++++	+++	−	−
48–23C	*C. parapsilosis*	−	−	++	++++	++++	++++	++++	++++	++−
48–23F	*C. parapsilosis*	−	−	−	++++	++++	++++	+++	+	−

was therefore conducted to determine the relative salt tolerance of the organisms isolated. Seventeen cultures showed a surprising tolerance to high concentrations of salt. (See TABLE II.) The isolates of *R. glutinis, R. mucilaginosa, R. peneaus, Trichosporon diddensii, Tr. lodderi, Candida guilliermondii* and *C. parapsilosis* were able to grow well in the presence of high concentrations of salt. The other isolates had a much lower tolerance to salt as is shown in TABLE II, and one isolate of *Tr. cutaneum* and the two of *Hansenula* were not able to grow in the presence of 8.3 per cent of salt. This is surprising, since species of *Hansenula* are usually tolerant to high concentrations of salt.

TEMPERATURE RANGE

Since much of the shrimp handled in Texas is frozen and in view of the recent observations of McCormack (**1950**) on the growth of a pink yeast at freezing temperatures, experiments were carried out to determine the temperature range for growth of the cultures isolated. (See TABLE III.) The *Trichosporon* species apparently are not well adapted to growth at low temperatures, but most of the cultures grow at 5° C. No significant growth occurred at 0° C., but several species of *Rhodotorula, Candida guilliermondii* and *T. aeria* showed appreciable growth at 2.5° C. when given sufficient time. Three isolates (*T. glabrata,* one strain of *C. parapsilosis* and *Tr. cutaneum* var. *peneaus*) were able to grow at 41.5° C., but none grew at 45° C. The isolate named *Rh. marina* had an exceptionally narrow growth range. It grew poorly

TABLE IV

RELATION OF TEMPERATURE TO THE SLANT COLOR OF CERTAIN ISOLATES

Code	Culture	Temperature and color	
48–23T	*R. glutinis*	5° C., bright yellow 10° C., dark yellow	14° C., salmon room temperature, pink
48–23W	*R. glutinis*	5° C., yellow 10° C., dark yellow	14° C., salmon room temperature, deep pink
48–23A	*R. peneaus*	5° C., cream	19° C. and above, canary yellow
48–23B	*R. peneaus*	5° C., cream	19° C. and above, canary yellow

at 10° C. and not at all at 32° C. If given more time at 14° and 19° C., the growth increased from moderate to heavy. It is a slow growing organism under the best of conditions.

Color of the streaks of certain cultures of *Rhodotorula* varied considerably with the temperature at which they were grown. These differences are shown in TABLE IV.

DISCUSSION

During the present study of yeasts associated with shrimps 35 cultures were isolated, of which eleven were *Rhodotorula*, nine *Trichosporon*, six *Candida*, three *Torulopsis*, two *Hansenula* and four *Pullularia*. Five new species and one new variety have been described: *Rhototorula peneaus, Rh. texensis, Rh. marina, Trichosporon diddensii, Tr. lodderi* and *Tr. cutaneum* var. *peneaus*.

Several noteworthy features have resulted from this survey. Presumably due to the fact that these yeasts were isolated from an environment lacking appreciable quantities of fermentable carbohydrates, nineteen cultures were entirely non-fermentative and the remaining fifteen, representing *Tr. diddensii, Tr. lodderi, C. guilliermondii, C. parapsilosis, T. glabrata* and *Hansenula californica*, were all weakly fermentative. It is also interesting that all cultures except two represented imperfect genera and that yeasts belonging to perfect genera such as *Saccharomyces, Debaryomyces* and *Pichia*, which are so commonly found in spoiled food, were not encountered. Red yeasts have been isolated from marine products and sea water a number of times and it is therefore not surprising that so many *Rhodotorula* cultures were isolated in this survey. Since these yeasts are also commonly found as air contaminants on plates, they might arrive in sea water from this medium. According to Zobell (**1946**, p. 178) many investigators have observed pink yeasts on plates exposed to marine air. On the other hand, it is possible for marine microorganisms to be released from sea water into the atmosphere by droplet formation during wave action, and some workers believe that their normal marine habitat is on certain species of algae.

As far as is known from the literature (see Lodder, **1934**), *T. albida* and *T. aeria* have also been isolated exclusively from the

air. The culture of *T. albida* isolated is in agreement with Lodder's description, even with respect to the weak yellow color. A petroleum ether or benzene extract of our isolate, as well as that of an authentic strain of *T. albida,* shows a yellowish-green color, indicating the presence of carotenoid pigments. There is undoubtedly a close relationship between *Rhodotorula* and this organism as well as several other weakly yellow, slimy, non-fermentative species of *Torulopsis.* However, until a study of the nature of these yellow carotenoids, now under way in our laboratory, has been completed, we hesitate to transfer *T. albida* into the genus *Rhodotorula.*

The pigments in *Rhodotorula pencaus* are carotenoid in nature, although they have not been fractionated and identified. Dr. Gordon Mackinney kindly ran absorption spectra on cultures *48–23A* and *B* and made the following comment: "These are somewhat different from *Rhodotorula* or *Sporobolomyces* pigments, particularly in the amount of alkali extractable ketonic carotenoids. Absorption on $MgO\text{-}SiO_2$ gave two distinct zones indicating at least two pigments. The petroleum ether extract had a slight greenish fluorescence indicating phytofluene."

Torulopsis glabrata has only been isolated thus far from human sources, where it is frequently found as a saprophyte and occasionally as a pathogen (compare Lodder and de Vries, **1938**, Benham, **1935**, and Black and Fisher, **1937**). It appears from their work that some strains are potentially pathogenic in susceptible individuals. Our isolate, which checks well with the description by Lodder and de Vries, is therefore the first case of its isolation from a different source.

The new *Trichosporon* species, *Tr. diddensii,* and *C. parapsilosis* appear to be related, since they have the same fermentation and assimilation characteristics. The important difference between the two species is the presence of arthrospores in the genus *Trichosporon.* It is interesting from an ecological point of view that representatives of both species were isolated from shrimp and, furthermore, that strain *48–23F* of *C. parapsilosis* has intermediate characteristics. This culture developed more true mycelium than the other cultures of *C. parapsilosis,* although arthrospores were not observed. Moreover, culture *48–23F* is arbutin-positive as is

Tr. diddensii, whereas the type culture of *C. parapsilosis* is arbutin-negative. This relationship between the two species is accentuated by the observation of Diddens and Lodder that their strain No. 2 of *C. parapsilosis* shows zigzag formation on pseudomycelium slides, a phenomenon which is very characteristic in the genus *Trichosporon*.

In comparing our results with the limited information in the older literature there is surprising agreement. Most of our isolates would have been termed red and white *Torulas*, with a negative or weak fermentation. Prior to this, *Trichosporon* species have not been isolated before from marine sources, but it is possible that in the older literature they may have been counted among the molds.

SUMMARY

1. Thirty-five cultures of yeasts were isolated from shrimp (*Peneaus setiferus*) collected in the Gulf of Mexico off the Coast of Texas.

2. Eleven of the cultures were members of the genus *Rhodotorula*, 9 of *Trichosporon*, 3 of *Torulopsis*, 4 of *Pullularia*, 6 of *Candida* and 2 of *Hansenula*.

3. Five new species and one new variety were described: *Trichosporon diddensii*, *Rhodotorula peneaus*, *R. texensis*, *R. marina*, *Trichosporon lodderi*, and *Trichosporon cutaneum* var. *peneaus*.

4. Salt tolerance of the isolates was determined by growth on wort agar with added sodium chloride. Appreciable differences in salt tolerance were noted.

5. The range of temperatures permitting growth for the various isolates was determined by incubating wort agar slants at the various temperatures for different periods of time. There were considerable differences in temperaure tolerances for growth between the different isolates. The color of certain cultures of *Rhodotorula* varied considerably with temperature of incubation.

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STUDIES ON VARIATION AND MUTATION IN ASHBYA GOSSYPII [1]

THOMAS G. PRIDHAM [2] AND KENNETH B. RAPER

(WITH 2 FIGURES)

Studies concerning mutation and variation in microorganisms have become increasingly important as their practical value in strain improvement of industrially-important fermentative microorganisms has been demonstrated. Yields greater than those obtained with parent strains may thus be realized, and in some cases the quality of the resultant fermentation product may be improved.

As early as 1935 mutation studies led to improved yields of economically important fermentation products. Kresling and Stern (**7**, **8**) reported the isolation of mutant substrains of *Aspergillus niger* capable of synthesizing considerably more citric acid than the parent strain. Since that time the itaconic acid fermentation (**9**), and polymyxin fermentation (**2**), and the streptomycin fermentation (**3**, **16**, **17**) have been improved by the isolation and selection of artificially induced mutant substrains. Most noteworthy has been the success achieved in improving the penicillin fermentation, where yields have been increased from less than 100 units per ml. to more than 900 units per ml. through selection of natural and radiation-induced mutant substrains of *Penicillium chrysogenum* (**1**, **4**, **5**, **6**, **11**, **13**).

Because of these developments, studies of natural and induced mutation have become accepted routines in the development of new industrial fermentations and in the improvement of established processes.

[1] Report of a study made under the Research and Marketing Act of 1946. Contribution from the Northern Regional Research Laboratory, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

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In an effort to improve the *Ashbya gossypii* process for riboflavin production, a detailed study of natural variation and induced mutation in this yeast-like fungus was made. No consistently superior riboflavin-producing substrains were obtained. However, a practical procedure for the evaluation of riboflavin production by substrains of the organism was developed, and interesting mutant types were isolated. Information obtained from these studies establishes a basis for future work in improving the process and for maintaining strains at peak riboflavin-synthesizing capacity. The present paper reports these results.

MATERIALS AND METHODS

The cultures studied consisted of the parent strain, *Ashbya gossypii* (Ashby and Nowell) Guilliermond, NRRL *Y-1056*, and the progeny resulting from the selection of naturally occurring variants and from the treatment of spores of this organism with various chemical and physical agents. A limited amount of work was done with other strains of *Ashbya* and species of the related genus, *Nematospora;* in no case, however, were yields of riboflavin in excess of 400 μg. per ml. obtained. Since this yield was substantially lower than that already being obtained with *A. gossypii*, NRRL *Y-1056*, attention was concentrated on this strain.

All cultures were maintained on Wickerham's M-Y medium, prepared as follows:

WICKERHAM'S M-Y MEDIUM

Difco Bacto peptone	0.5%
Difco Bacto yeast extract	0.3%
Difco Bacto malt extract	0.3%
Commercial glucose (Cerelose, Clintose) [3]	1.0%
Difco Bacto agar	2.0%
Distilled water	

Do not adjust pH, which is about 6.00 after sterilization. Sterilize at 121.5° C. for 20 minutes.

Cultures were incubated continuously at 27 to 30° C. and were transferred weekly to fresh agar slants. During the later phases

[3] The use of trade names is for information only and does not constitute an endorsement of these products.

of the study, cultures were incubated at 27 to 30° C. for two days, followed by completion of the incubation cycle at 25 to 26° C.

The M-Y medium was presumed to contain all the necessary requirements for optimal growth of the parent strain, hence it served as the complete medium in the physiological studies and was the medium of choice for the cultural and morphological work. A minimal medium, presumed to supply only those factors absolutely necessary for the growth of the parent strain, was based on the biotin assay medium of Robbins and Schmidt (**15**) and Robbins (**14**) in which *A. gossypii* gives graded responses with biotin. It was prepared as follows:

ASHBYA MINIMAL MEDIUM

Difco Bacto dextrose	5.0%
Difco Bacto asparagine or $(NH_4)_2SO_4$, C.P.	0.2%
KH_2PO_4, C.P.	0.15%
$MgSO_4 \cdot 7H_2O$, A.R.	0.05%
Difco Inositol	0.02%
Thiamin hydrochloride, U.S.P.	50.0 μg.%
d-Biotin, Hoffman-LaRoche	1.0 μg.%
Difco Bacto agar	1.5%
Distilled water	

Adjust pH to 6.5 and sterilize at 121.5° C. for 20 minutes.

For production of spores, Gorodkowa's ascospore medium in liquid form was found most suitable. It was prepared as follows:

GORODKOWA'S ASCOSPORE MEDIUM

Commercial glucose	0.25%
Difco Bacto beef extract	1.00%
NaCl, A.C.S.	0.50%
Distilled water	

Do not adjust pH. Dispense in 100-ml. quantities into 1-liter Erlenmeyer flasks, and sterilize at 121.5° C. for 20 minutes.

For evaluating riboflavin production by the various substrains, considerable emphasis was placed upon establishing a procedure whereby maximum yields and reproducibility could be obtained. A satisfactory procedure was developed requiring two media, an inoculum broth and a fermentation medium.

The inoculum broth for bringing cultures to the proper physiologic state for riboflavin synthesis was prepared as follows:

INOCULUM BROTH

Commercial glucose	2.0%
Difco Bacto peptone	0.5%
Corn steep liquor (wet basis) [4]	2.5%
K_2HPO_4, C.P.	0.1%

Dissolve ingredients in distilled water, adjust to pH 6.60 to 6.80 with KOH, dispense in 10-ml. quantities into 50-ml. Erlenmeyer flasks, and sterilize at 121.5° C. for 20 minutes. Following sterilization, remove flasks from autoclave immediately.

The fermentation medium was similar in composition to the inoculum broth except that the glucose concentration was increased to 4%. It was dispensed in 100-ml. quantities into 500-ml. Erlenmeyer flasks, and sterilized in the same manner. In both these media, a heavy precipitate formed upon adjustment of the pH to the proper range, necessitating thorough mixing while dispensing.

All liquid cultures were incubated with agitation at 27 to 30° C. on a reciprocal shaker operating at 80 strokes per minute (3-inch stroke).

Substrains for studies of natural variation were obtained from routine platings of spores in M-Y medium incubated for 4 to 5 days at 27 to 30° C. Clones were picked at random and transferred to slants of M-Y medium for subsequent study.

In attempts to induce mutation in the organism, spore suspensions were prepared by cultivating the organism in Gorodkowa's medium for 5 to 6 days. At the end of the incubation period, the culture was agitated with sterile sand, filtered through cotton, and the resulting suspension, consisting almost entirely of spores, was then centrifuged and diluted in the appropriate medium to the concentration desired.

[4] Study of a number of lots of corn steep liquors indicated that the concentration of this ingredient in the inoculum and fermentation media depends to a great extent upon the particular lot employed. Thus, with each batch of corn steep liquor it is necessary to redetermine optimal concentrations. The corn steep liquor most used in the present study was described as a heavy incubated steep water obtained shortly after concentration. Its characteristics were: solids, 54.6%; pH, 4.44; sp.g., 1.23; free sugar, 1.64%. Total N, 4.32%; amino N, 1.40%; and ammonia N, 0.042%. It was obtained from the Corn Products Refining Company, Argo, Illinois, in 1945 and has been stored under refrigeration since that time.

TABLE I lists the conditions under which spores were subjected to the various agents. The ultraviolet source was the germicidal lamp described by Raper and Fennell (**12**). During irradiation spores were suspended in water, or some liquid medium, and contained in a flat-bottomed Petri dish to a depth of about 5 mm. Suspensions were stirred continually with a small glass rod. Aliquots were removed at regular intervals, diluted, and plated in M-Y medium. The authors are indebted to Dr. N. N. Hellman of the Analytical and Physical Chemistry Division of this Laboratory for valuable assistance in carrying out the X-radiations. In these ex-

TABLE I

CONDITIONS UNDER WHICH SPORES OF *Ashbya gossypii* WERE EXPOSED TO MUTAGENIC OR SELECTIVE AGENTS

Agent	Suspending medium	Spore concentration/ml.	Distance from radiation	Time of exposure
			cm.	
Ultraviolet radiation: 4-watt General Electric lamp emitting 500 milliwatts radiation at 2537 Å. (95% total radiation)	27 ml. saline	60,000	10.8	0–15 min.
	25 ml. Gorodkowa medium	90,000	10.8	0–15 min.
	30 ml. distilled water	20,000	10.8	0–15 min.
	27 ml. saline	80,000–100,000	23.5	0–15 min.
X-radiation: 15 ma. unfiltered Cu radiation, 38 Kv.	*ca.* 120,000 spores on surface of complete medium.		29.0	0–120 min.
CH_3-bis-β-chloroethyl-methylamine hydrochloride 1%	30 ml. citrate-PO_4 buffer, pH 6.0	125,000	—	0–30 min.
0.2%	10 ml. 2.7% $NaHCO_3$	*ca.* 1,000,000	—	0–30 min.
Sodium thiosulfate 0.025–4.0%	In M-Y medium	loop dilutions	—	4–5 days
Sodium dithionate 0.10–2.50%	In M-Y medium	loop dilutions	—	4–5 days
Sodium dithionite 0.10–2.50%	In M-Y medium	loop dilutions	—	4–5 days
Neutral red (Bacto) 0.002–1.0%	In M-Y medium	loop dilutions	—	4–5 days

periments it was necessary to shield half of each plate surface with lead in order to obtain optimal radiation effects. Spores exposed to X-radiation were spread over the surface of M-Y medium prior to exposure. Following treatment with the nitrogen-mustard compound (CH_3-bis-β-chloroethyl-methylamine hydrochloride),[5] aliquots were inactivated by incubation in a glycine-$NaHCO_3$ buffer (glycine, 0.3 gms.; $NaHCO_3$, 0.34 gms.; distilled water, 500 ml.)

[5] The authors are indebted to Dr. R. Macy, Chief, Chemical Division, Chemical Corps Technical Command, Army Chemical Center, Edgewood, Maryland, for kindly supplying the nitrogen-mustard compound for these experiments.

for 2½ to 3 hours, after which they were appropriately diluted and plated in M-Y medium. Other chemical agents tested were added to M-Y medium in the desired concentration, sterilized, and dilution plates made with spore suspensions.

The inoculated media were incubated for 4 to 5 days at 27 to 30° C., at the end of which time random clones were picked to slants of M-Y medium and held for subsequent study.

Cultural characteristics of each substrain were determined from daily observations of "spot-inoculated" colonies growing on M-Y medium. Colony diameters were measured to determine relative growth rate, and the number of days required for the development of pigment and the nature of the pigment on the tenth day of incubation were recorded. Other cultural characteristics such as the nature of the advancing edge of the colony, presence or absence of sectors, or of spiny outgrowths (funiculation), and the general topography of the colony, i.e., if it was wrinkled, radially ridged or grooved, flat, smooth, or compact, were observed and recorded.

The morphological characteristics of each substrain were studied with preparations from the 10-day colonies on M-Y medium. Flat wet mounts were prepared by cutting a radial section (approximately 2 mm. wide) from each colony. Agar adhering to the reverse of the section was pared away, the bit of growth placed on a microscope slide, one drop of distilled water added, and the preparation gently crushed beneath a cover slip. These were examined at 200 or 300 diameters magnification. Spore, hyphal, and sporiferous sac (pro-asci or pro-sporangia) dimensions were measured; relative numbers of spores, sporiferous sacs, and bulb-forms were determined; and the nature and type of hyphal branching and the nature of the sporiferous sacs were recorded (see Pridham and Raper, (**10**), for detailed discussion of morphological characteristics).

In the study of the physiology of the substrains, each was tested in regard to its ability to grow on the minimal media and compared with the parent strain in its ability to synthesize riboflavin.

To determine if any substrain was characterized by a nutritional deficiency, each was transferred to slants of both asparagine minimal and ammonium sulfate minimal media. The inoculum consisted of

growth from 7-day stock cultures. In order to obviate carry-over of nutrient material, no visible inoculum was left on the surface of the inoculated slants. All cultures were incubated for 5 days at 27 to 30° C. At that time each culture was examined for the presence or absence of growth or any other notable characteristic. Those substrains showing obviously altered growth habits on these media were retested at least once and in many cases, several times. Substrains evidencing continued altered growth habits on these media were recorded as physiological mutants. These were later studied more intensively by culturing in the minimal media supplemented with various complex nitrogenous materials, vitamins, amino acids, and purine and pyrimidine bases, added singly or in combination. Media for these tests were prepared in liquid form, dispensed in 10-ml. quantities into 50-ml. Erlenmeyer flasks and

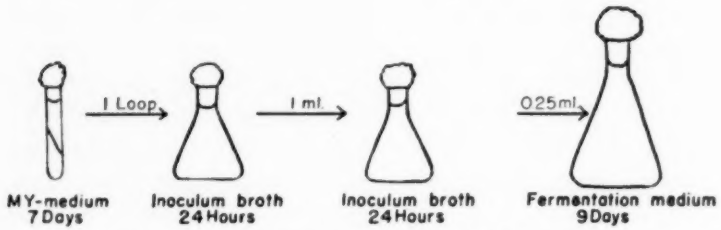

FIG. 1. Procedure for conducting riboflavin fermentations.

sterilized. Cultures were incubated with agitation for 7 days at 27 to 30° C. and the relative amount of growth and any other notable characteristic recorded.

The ability of each substrain to synthesize riboflavin was compared with that of the parent strain by conducting fermentations by the standardized procedure developed in the earlier phases of the investigation (FIG. 1). In developing this procedure a number of changes were made in the original methods employed for producing riboflavin in the laboratory. To improve reproducibility of results, peptone was resubstituted for the animal stick liquor previously employed as a source of nitrogen. Corn steep liquor concentrations were increased, and a rigid schedule for handling of stock cultures was instituted. Two significant changes which resulted in increased yields of the vitamin involved passage of the organism

through an additional broth culture prior to inoculation of the fermentation medium, and the use of a small volume of inoculum (one-fourth of a ml. per 100 ml. of fermentation medium). With the above changes in procedure, yields of riboflavin of the order of 1000 μg. per ml. of culture liquor were regularly obtained with the parent strain. Fermentations were conducted at 27 to 30° C. with agitation for 9 days.

On the seventh and ninth days of fermentation, approximately 15 ml. of fermented liquor was removed from each flask of fermentation medium. Ten ml. of this sample was placed in a 100-ml. volumetric flask, 1 ml. of acetate buffer (pH 4.5 to 4.7) added, and the buffered sample hydrolyzed for 30 minutes at 121.5° C. The hydrolyzed sample was appropriately diluted with distilled water and assayed fluorometrically at 4360 Å. (Coleman Electronic Photofluorometer, Model 12) by comparison with the fluorescence of Hoffman-LaRoche riboflavin (0.4 μg./ml.). Microbiological assays gave equivalent results when compared with this method of assay. The remainder of the 15-ml. sample was used in determining the pH of the fermenting culture.

EXPERIMENTAL RESULTS

728 substrains were examined. None of those obtained from routine platings in M-Y medium (possible natural variants) differed markedly from the parent strain (FIG. 2A; see description in Pridham and Raper (**10**)).

Description of mutant substrains

In general, most of the isolated substrains obtained by treatment with the various agents closely resembled the parent. However, a substantial number were characterized by apparent heritable alterations.

Stable mutant substrains were obtained through the use of both ultraviolet and X-radiation. These could be categorized as follows:

a) Substrains with hyphae thinner than normal, or aberrant.

b) Substrains with depressed or complete inability to form spores.

c) Substrains with altered morphology of pro-asci (pro-sporangia) or complete inability to form these cells.

d) Substrains showing less total growth than the parent.

e) Substrains showing altered or delayed pigmentation.

f) Substrains forming compact, mold-like colonies (FIG. 2B).

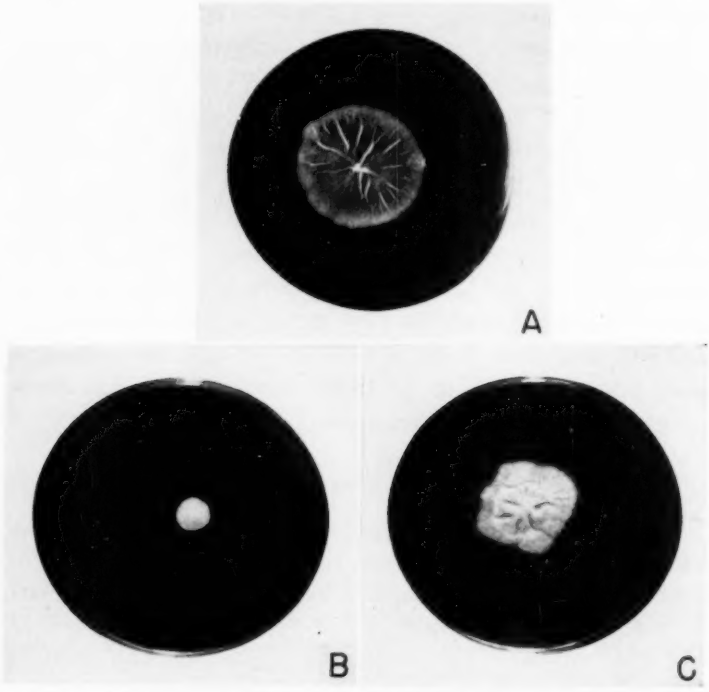

FIG. 2. *Ashbya gossypii* (Ashby & Nowell) Guillierm. Ten-day colonies growing on M-Y medium at 27° to 30° C. A. Strain NRRL *Y-1056* (parent). B. Ultraviolet mutant forming compact colony. C. Ultraviolet mutant characterized by non-uniform growth rate.

g) Substrains forming pasty, yeast-like colonies.

h) Substrains forming colonies with increased tendency to sector.

i) Substrains showing non-uniform growth rates (FIG. 2C).

j) Substrains with very limited vitality, dying out after several transfers on M-Y medium.

Some of these phenomena were also exhibited as temporary

changes, since repeated sub-culture on M-Y medium led to reversion in the direction of the parent strain. In many cases, multiple changes were evident in single substrains. Thus, one isolate obtained from ultraviolet treatment was consistently unable to form sporiferous sacs or spores, produced hyphae approximately 5 μ in width rather than the normal 6–10 μ, produced no bulb-forms, synthesized no measurable amount of riboflavin, and gave only faint growth in the minimal media. Other mutants of this general type were isolated and have remained stable over a period of several years with routine weekly or monthly sub-culture on M-Y medium. Two substrains were isolated which possessed temporary ability to synthesize limited amounts of riboflavin in the minimal media. The parent strain is unable to effect synthesis of noticeable amounts of the vitamin in these media.

Several substrains obtained by X-radiation were of interest because they possessed characteristics closely approaching those of various species of *Nematospora*. Colonies were of the same paste-like consistency, and examination indicated the morphology of the cells to be the typical yeast-like form. This type of mutant was not observed in survivors of any other treatments.

Mutant types showing cultural characteristics similar to those obtained with ultraviolet and X-radiation were obtained following treatment with the nitrogen-mustard compound. However, the most interesting types were those characterized by nutritional deficiencies. No other treatment led to the isolation of stable deficiency mutants. Four of these substrains were characterized by their inability to grow in either the asparagine or ammonium sulfate minimal media. Their exact nutritional requirements have not been conclusively established, but they appear to require mixtures of factors present in the peptone, yeast extract, and malt extract of the M-Y medium. Despite these nutritional deficiencies, these substrains are still capable of synthesizing moderate amounts of riboflavin.

Neutral red, sodium thiosulfate, sodium dithionate, and sodium dithionite were studied, not for their possible mutagenic activities, but because of their potentialities as indicators for the selection of highly flavinogenic substrains from a population presumably heterogeneous with regard to vitamin-producing capacity. These

materials, with the exception of neutral red, have been used in the isolation of riboflavin from fermented liquors because of their ability to reduce the vitamin to precipitable compounds, characterized by altered color. Thus, under suitable conditions, riboflavin should be reduced and colony pigments altered. Those colonies in which little or no alteration in pigmentation was noticeable would presumably have synthesized sufficient riboflavin to overcome the effects of the particular concentration of reducing agent present. Selection of such colonies would aid in maintaining highly flavinogenic strains, or possibly in improving strains.

Three pigmented types of colonies appeared following inoculation of M-Y medium supplemented with Bacto neutral red. They were pink, reddish-orange, and orange-yellow. The indicator was not appreciably toxic to the organism in the lower concentrations.

Under the experimental conditions, fairly low concentrations of sodium thiosulfate or sodium dithionate (0.025 to 0.5%) appeared to enhance the pigmentation of surviving colonies. As will be indicated later, many clones with enhanced pigmentation subsequently gave higher yields of riboflavin than the parent strain in comparative fermentations. In the higher concentrations, there was a greater percentage of clones characterized by depressed pigmentation. Sodium dithionite proved to be toxic in all concentrations tested, no colonies developing on media supplemented with this material. Sodium dithionate proved less toxic than sodium thiosulfate, which inhibited growth as shown by decreasing colony counts with increasing concentration. There was no evidence that sodium dithionate was toxic at levels of as much as 2.5%. However, with increasing concentrations of this material, a progressive increase in the number of white colonies was noted. Many of the lesser pigmented colonies on media containing 1.5% or more of this compound were culturally aberrant, being ragged and compact. A few of these remained stable during the remainder of the study.

Riboflavin synthesis by mutant substrains

The abilities of the various substrains to synthesize riboflavin in laboratory fermentations were tested over a period of 3 years. In general, the substrains could be separated into three categories on the basis of their flavinogenic abilities:

a) Substrains synthesizing considerably less riboflavin than the parent strain.
b) Substrains synthesizing riboflavin in amounts essentially equivalent to those of the parent strain.
c) Substrains synthesizing riboflavin in amounts of the order of 100 to 200 μg. per ml. more than the parent strain in comparative fermentations.

The majority of the substrains which possessed obviously altered morphological and cultural characteristics were in the first category, while the second and third categories contained substrains outwardly similar to the parent.

Those substrains which showed promise of increased flavinogenic capacity in the early stages of the work progressively lost their abilities to synthesize more riboflavin than the parent. The same phenomenon of gradual degeneration, though less marked, has been noted with the parent strain. However, this tendency toward lessened flavinogenesis in the parent strain can be prevented by careful maintenance of stock cultures. Whereas mutant substrains were observed to produce 100 to 200 μg. per ml. of riboflavin more than the controls in particular experiments, the total yields produced by such mutants did not exceed yields obtained from the parent strain in other isolated experiments.

An attempt was made to correlate pigmentation of the colony with subsequent riboflavin production in the case of those clones obtained from M-Y media supplemented with sodium thiosulfate, sodium dithionate, or neutral red. Yields of the same order resulted with both the white and orange-yellow colonies obtained by the use of sodium thiosulfate. Many of these exceeded the parent in comparative tests, but again by no more than the limits of variation experienced with the parent strain. However, the majority of the white or pale yellow colonies obtained with sodium dithionate, and the pink and reddish-orange colonies isolated from the neutral red media, proved inferior to the parent strain. It was later found that the incorporation of 0.1% sodium dithionate in M-Y medium led to enhanced pigment production, and fermentations originating from stock cultures on this medium gave greater yields of riboflavin than the parent strain in comparative tests. Supplementation of the fermentation medium with 0.1% sodium dithionate and

use of inocula prepared from cultures maintained on the regular M-Y medium gave similar increases in yield. However, when dithionate was added to both stock culture media and fermentation media, subsequent fermentations were no better than control fermentations. Neutral-red substrains marked by orange-yellow pigmentation generally gave higher yields than the parent strain in comparative tests, but again these yields were of the same order as those obtained with other substrains.

DISCUSSION

The original intent of this study was to determine if increasing the rate of variation or mutation in *Ashbya gossypii* NRRL *Y-1056* might improve the chances of securing a substrain or substrains marked by substantially increased flavinogenesis. Unfortunately, no substrains were isolated which proved significantly superior to the parent strain in repeated comparative tests. These results do not necessarily imply that the isolation of stable superior strains of *A. gossypii* is not possible. Indeed, in other investigations, the isolation of superior fermentative substrains has required the testing of considerably more isolates than were tested in this study. Thus, Savage (**16**) found that only 3 of 3700 strains of *Streptomyces griseus* isolated and tested were stable superior streptomycin producers. In our study of slightly more than 700 isolates, the possibilities of obtaining superior substrains would seem to be limited.

The fact that the substrains obtained by routine dilution plates showed little, if any, tendency toward heritable alterations is good evidence that the mutagenic agents employed increased the rate of mutation. This is further substantiated by the observation that the majority of heritable changes were of a degenerate nature not observed at any time in normal strains of the organism. Complete genetic analyses of the alterations noted are impossible because the organism manifests no well-defined sexual phase. Hybridization in this species is not technically possible, as this fungus appears to be homothallic, and fusion of "ascospores" has not been observed.

Obvious mutants were obtained among the survivors of all radiation exposure periods. Had survivors been picked only from those

plates showing 90% or more killing, many of these mutant types would have been missed. Since the primary interest was in the mutants themselves, no attempt was made to obtain statistically accurate data on mutation frequencies.

The limited tendency of untreated substrains to form sectors of less intense pigment probably indicates an inherent tendency for variation to a non-flavinogenic form. This is further substantiated by the observation that, rather infrequently, the parent strain appears to lose some of its capacities for prodigious riboflavin synthesis. Such a tendency appears spontaneously, lasts for a short time, and then disappears.

There is good evidence that the parent strain can vary in the opposite direction of increased flavinogenic capacity. Observation of this phenomenon led to Wickerham's discovery of strain NRRL *Y-1056* as a potential industrial riboflavin-producing organism (**18**). A few of the untreated substrains in this study were found to synthesize more riboflavin than the parent in comparative tests. Eventually their capabilities decreased until they produced no more than the parent.

Little or no correlation between altered characteristics and increased riboflavin production was observed. It was noted, however, that substrains exhibiting altered cultural and morphological characteristics generally failed to produce large quantities of the vitamin. Those substrains characterized by inability to form sporiferous sacs or spores, and those forming compact colonies (FIG. 2B) or colonies with ragged, uneven, advancing edges (FIG. 2C), exhibited greatly retarded flavinogenic capacities. These results would seem to indicate that the over-all trend of effects of the mutagenic agents was toward decreased tendencies to synthesize the vitamin.

Variation in flavinogenic capacity, particularly in the direction of decreased flavinogenesis, indicates the necessity for proper maintenance of stock cultures of the organism. The incorporation of certain oxidation-reduction compounds, such as sodium dithionate, in culture media represents a means for attaining this end. Frequent reisolation of superior substrains from the parent, or immediate lyophilization or preservation under oil of proved highly flavinogenic substrains is likewise indicated.

The current investigations have led to substantial improvement in the conduct and reproducibility of the riboflavin fermentation with *A. gossypii*. Nevertheless, variations of 200 to 300 μg. of riboflavin per ml. are still occasionally obtained in replicate fermentations. The need for further improvement is obvious. Some of the inconsistencies previously experienced are believed to have been obviated by replacing extremely variable nitrogenous constituents of the fermentation medium with peptone as originally employed by Wickerham *et al.* (**18**). Yields have been increased by progressively increasing the concentration of selected corn steep liquors and by reducing the volume of specially prepared inocula. From results obtained in this phase of the investigation, it appears that the peptone contributes primarily to the growth of the organism, and that the corn steep liquor is a critical component leading to high yields of the vitamin. If this be true, further analyses of these materials might lead to methods for the standardization of media ingredients, and might reveal the critical compounds for riboflavin synthesis.

In the investigations concerned with the development of a satisfactory procedure for testing flavinogenic capacities of the substrains, it was found that the volume of inoculum required depended upon its method of preparation. The procedure for conducting fermentations, illustrated in FIG. 1, was adopted as a result of this work. Information obtained indicates that the most critical stage of the fermentation occurs during the first 36 hours and is influenced to a considerable extent by the inoculum. If the organism is capable of combating the initial acidity formed during this period, either through the production of neutralizing substances or by utilization of the acids formed, a satisfactory fermentation will result. If not, an abnormal fermentation, in which the pH remains at a low level and little or no riboflavin is produced, will occur. The abnormal fermentations which are encountered from time to time, in both laboratory and large-scale operations, are of considerable concern. The explanation for their occurrence is still problematical. Preliminary studies employing paper chromatography have indicated that normal and abnormal fermentation liquors differ qualitatively in their content of organic acids. Further study along these lines would be of considerable interest and might give

information concerning the mechanisms of both types of fermentations.

Critical examination of various inocula leading to normal fermentations with high yields of riboflavin showed these to possess certain characteristics. The organism was present entirely in the vegetative state, i.e., no spores or sporiferous sacs were present; the pH range was between 5.70 and 6.80; and usually some initial riboflavin synthesis had occurred in the second of the two successive 24-hour inoculum broth cultures. In addition, these inocula were invariably homogeneous, which greatly facilitated their handling.

The primary objective in undertaking this phase of the study was to secure reproducibility of results for the purpose of comparing flavinogenesis in the parent strain and in mutants derived therefrom. Emphasis was not placed upon the attainment of maximal yields. It has already been reported that riboflavin can be produced in amounts up to 1760 μg. per ml. by supplemental feeding of *A. gossypii* during the fermentation (**10**). The potentialities of this organism as a source of riboflavin are well established.

ACKNOWLEDGMENT

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SUMMARY

The spores of *Ashbya gossypii*, NRRL *Y-1056*, have been treated with ultraviolet radiation, X-radiation, and CH_3-bis-β-chloroethylmethylamine hydrochloride. The general tendency was for these mutagenic agents to induce the formation of degenerate types with greatly retarded flavinogenic abilities. Limited evidence of natural variation was obtained in the study. Some treated substrains were characterized by an unsustained ability to produce greater amounts of riboflavin than the parent. The desirability of adequate maintenance of stock cultures was demonstrated. The addition of sodium dithionate to stock culture media in 0.1% concentration

enhanced pigment formation and tended to maintain the organism in its highly flavinogenic state.

A practical procedure for the evaluation of riboflavin production by substrains of *A. gossypii* was developed. By employing peptone and increased concentrations of selected corn steep liquor in fermentation media, by careful attention to stock cultures, and by critical control of inoculum preparation and use, yields of the order of 1000 μg. of riboflavin per ml. of fermentation medium were regularly obtained with the parent strain.

The results of this study have given a somewhat clearer picture of the actual potentialities of *A. gossypii* as a source of riboflavin and further indicate its excellence as a microbial producer of this vitamin. Variations still encountered in replicate fermentations, and the occurrence of both abnormal and normal fermentations in replicate flasks, indicate a possibility for added improvement in procedures.

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CULTURAL AND NUTRITIONAL STUDIES OF TRICHOPHYTON GALLINAE AND TRICHOPHYTON MEGNINI

LUCILLE K. GEORG

(WITH 5 FIGURES)

During the past 40 years there has been considerable confusion in the literature of medical mycology concerning the true identity of the organism here referred to as *Trichophyton gallinae* and *Trichophyton megnini* Blanchard 1896. These organisms have several cultural characteristics in common, and Sabouraud equated them in his early studies. Although by 1910 he had revised his opinion and, in Les Teignes (**22**), carefully described the two as distinct species, the confusion has persisted to the present time.

It was considered advisable, before beginning experimental studies with strains of these fungi, to review their history and classification so that there would be no doubt as to the identity of the species involved. The first reference to *T. gallinae* is that by Mégnin who, in 1881 (**15**), described a disease in a cock that was characterized by "a fine moldiness on and about the comb and cheeks." Scrapings showed mycelia and chains of round spores 6 to 8 μ in diameter (probably arthrospores). Mégnin stated that the fungus multiplied exclusively on and within the scales of the epidermis and did not enter the feather follicles or quill. He proposed the name *Epidermophyton gallinae* for the fungus that caused this disease; although at this time he had not isolated the organism nor described it in culture.

The valid description of the fungus based on cultures did not appear until 1890 (**16**) when Mégnin presented and described cultures, which had been isolated from diseased chickens. His description of the snow-white colony with the striking current-red pigment which diffused throughout the medium makes it clear that he had the same culture which Constantin and Sabrazès, 1893 (**6**),

Blanchard, 1896 (**3**), Matruchot and Dassonville, 1899 (**14**), and later Sabouraud, 1910 (**22**) studied in more complete detail.

The first description of *T. megnini* was given by Sabouraud in 1893 (**20**), when he designated it simply as the "trichophyton à culture rose," without supplying a binomial. He had isolated the fungus from human lesions which were described as "ichthyosis pilaire" or "trichophyton sèche" with hairs invaded in an "ectothrix manner." The cultural description of the "Trichophyton à culture rose" was very clear and there is no doubt that the fungus he described then was identical with the one later named *T. megnini* by Blanchard. The colony was slow-growing, the surface snow-white and velvety when young, and heaped like a small powder puff. As the culture aged, it flattened into a disc-like colony and developed fairly regular radial grooves. The surface gradually became a pale rose and finally a deep violet-currant color. A characteristic currant-red pigment was visible on the reverse side of the colony but did not diffuse into the medium beyond the limits of the colony. On the conservation medium of Sabouraud (**22**) (a simple 1 percent peptone medium without sugar) the characteristic red pigment was not produced, but one could see a dark purple or black spot, the so-called "tache d'encre" of Sabouraud, in the center of the reverse side of the colony.

Shortly after Sabouraud's first description of the "trichophyton à culture rose," Mégnin sent him the head and neck of a chicken which had shown signs of the disease Mégnin had described in 1881. The culture from this chicken and others described by Duclaux from similarly diseased chickens, were considered by Sabouraud to resemble those of his "trichophyton à culture rose," and he stated that the fungus which caused the disease in man and which he had described in 1893 was the causative agent of the disease described by Mégnin in chickens. This belief was strengthened by the fact that in the first two human cases which he had encountered there was some evidence that the infection had been contracted from birds.

In 1896, however, on the basis of clinical and bibliographical studies, Blanchard (**3**) clearly distinguished the two species and described them separately. For the fungus isolated from the

chicken by Mégnin he retained the name *Epidermophyton gallinae*, but for the "Trichophyton à culture rose" of Sabouraud he proposed the name *Trichophyton megnini*. It is unfortunate that he named this latter fungus after Mégnin, because it is clear from his cultural description that he was referring to the organism which Sabouraud had isolated from human infections.

In 1902 Bodin (**4**), influenced by Sabouraud's confusion of the two species, considered the fungus of Mégnin to be identical with that of Sabouraud and gave the name *Trichophyton roseum* to the combined species, listing *T. megnini* Blanchard as a synonym.

To add to the confusion, Sabouraud, in 1909 (**21**), disregarded both the names *T. megnini* of Blanchard and *T. roseum* of Bodin and gave the name *Trichophyton rosaceum* to his "trichophyton à culture rose." In Les Teignes, 1910 (**22**), he made clear that he had been in error in identifying this organism with *E. gallinae* of Mégnin, but he stated that both Blanchard and Bodin had been influenced by his early error and that therefore the names which they proposed were invalid.

It is possible that Blanchard was confused regarding these two species, and indeed in the last paragraph of his description of *T. megnini* he says that there is evidence that this fungus is transmitted to man from birds, stating that Sabouraud and Mégnin studied it in the hen. In spite of this fact, the two species are distinctly and separately described in the Blanchard reference and the name *T. megnini* Blanchard must be considered valid for the "Trichophyton à culture rose" isolated and described by Sabouraud in 1893.

In Les Teignes 1910 (**22**) Sabouraud retained the species name *gallinae* for the fungus of Mégnin, but he had come to the conclusion that the lesions produced by this organism were not of the ringworm type, but were of a favic nature, and therefore placed this organism in the genus *Achorion* making the new combination *Achorion gallinae*. He described *A. gallinae* here as the *E. gallinae* of Mégnin, a fungus which causes a natural infection in chickens and turkeys, producing a mycosis of the favic type. On one occasion it had been inoculated experimentally into man, and he cited one instance where it had been obtained from a spontaneous ring-

worm lesion of man. In Les Teignes we have a clear description of this fungus. It was described as a mold which grew more rapidly than *T. megnini* (*T. rosaceum*) and produced at first a rather flat colony with a white, downy to fluffy surface growth. Later the culture developed irregular radial grooves and the surface gradually became a delicate pink. The most striking feature, however, was the production of a soluble strawberry-colored pigment which diffused readily into the medium, first forming a halo about the colony and later coloring the entire medium a deep strawberry color. Old colonies might become somewhat powdery and develop cracks on the crests of the folds. On media without sugar, such as the conservation medium of Sabouraud, no pigment was produced.

Unfortunately, this species has been shifted to several other genera in the course of the development of the classification of the dermatophytes. Most of these genera have been found to be invalid and will not be reviewed here, although they are cited in the list of synonyms given below. Both microconidia and macroconidia were described for this fungus by Sabouraud in Les Teignes (**22**). He stated that the fuseaux (macroconidia) were quite rare, but were dispersed throughout the culture. He sketched them in this text (p. 708) as slender, slightly clavate, thin-walled structures with 2 to 6 cells. The microconidia he described as rare, delicate, and well-spaced along the mycelium. Baudet (**2**) has also described the spores of this species. In typical cultures of *T. gallinae* isolated from a dog which had been confined to a small enclosure where chickens previously had been kept, he observed microconidia as well as numerous fuseaux (macroconidia). The macroconidia as illustrated were several-celled, clavate, thin-walled structures. The author stated that from the botanical point of view there could be no objection to the classification of this fungus in the genus *Trichophyton*. These structures have also been observed in the several strains included in the present studies, and photomicrographs and camera lucida drawings have been made. On the basis of these studies, and the classification of the dermatophytes as suggested by Emmons (**8**), it is proposed that the correct name for the fungus of Mégnin be established as *Trichophyton gallinae*.

LIST OF SYNONYMY

TRICHOPHYTON MEGNINI Blanchard, 1896 (3)

Trichophyton à culture rose Sabouraud, 1893 (20)
Trichophyton roseum Sabouraud apud Bodin, 1902 (4)
Trichophyton rosaceum Sabouraud, 1909 (21)
Trichophyton vinosum Sabouraud, 1910 (22)
Ectotrichophyton megnini Castellani & Chalmers, 1919 (5)
Megatrichophyton megnini Neveu-Læmaire, 1921 (17)
Sabouraudites megnini Ota & Langeron, 1923 (18)
Aleurosporia rosacea Grigoraki, 1925 (11)
Megatrichophyton roseum Dodge, 1935 (7)

Trichophyton gallinae (Mégnin) n. comb.

Epidermophyton gallinae Mégnin, 1881 (15) (nomen nudum)
Epidermophyton gallinae Mégnin, 1890 (16)
Lophophyton gallinae Matruchot & Dassonville, 1899 (14)
Achorion gallinae Sabouraud, 1910 (22)
Sabouraudites gallinae Ota & Langeron, 1923 (18)
Closteroaleurosporia gallinae Grigoraki, 1925 (11)
Microsporum gallinae Grigoraki, 1929 (12)
Sabouraudites gallinae Langeron & Milochévitch, 1930 (13)

MATERIALS AND METHODS

Studies were undertaken to determine the requirements for optimum growth and spore production in a series of strains of *T. megnini* and *T. gallinae*. A list of these strains with their sources and a description of their colonial appearance after 4 weeks' growth on Sabouraud dextrose agar (Difco) are presented in TABLES I and II.

A basal vitamin-free medium was prepared as follows: 50 g. dextrose, 0.5 g. $MgSO_4$ and 0.5 ml. trace mineral solution [1] were placed in a liter of distilled water buffered to pH 7.2 with Sorensen's phosphate mixture (KH_2PO_4 and Na_2HPO_4). For solid media, 1.5 percent washed, vitamin-free agar was added. Various

[1] H_3BO_3—28.5 mg.; $CuSO_4 \cdot 5H_2O$—93.0 mg.; MoO_3 (85%)—17.6 mg.; $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$—865.0 mg.; $MnSO_4 \cdot H_2O$—30.5 mg; $ZnSO_4 \cdot 7H_2O$—395.0 mg.; H_2O dist. to 500 ml.

TABLE I

CHARACTERISTICS OF *Trichophyton megnini* STRAINS ON SABOURAUD DEXTROSE AGAR

Culture identification as received	Source	CDC No.	Description of colony on Sabouraud dextrose agar after 4 weeks
T. rosaceum (strain Gaillard)	R. W. Benham No. 299–11 (Sabouraud's Collection, Paris)	424	Flat with fairly regular radial grooves. Border sometimes irregularly scalloped. Surface downy or velvety, whitish in center, but mostly a deep rose. Submerged growth around edge a dark rose, giving a dark border to colony. Reverse is a very deep rose.
T. rosaceum (strain Vrijman)	R. W. Benham No. 361 (Central Bureau of Fungus Cultures, Netherlands)	425	Similar to No. 424.
T. megnini (strain C.B.S.)	Central Bureau of Fungus Cultures, Netherlands	A-299	Similar to No. 424.
T. megnini (strain Blank)	Central Bureau of Fungus Cultures, Netherlands	A-302	Similar to No. 424.

TABLE I—*Continued*

Culture identification as received	Source	CDC No.	Description of colony on Sabouraud dextrose agar after 4 weeks
T. rosaceum	N. F. Conant, Duke University	359	Slower growing than above, producing a small, heaped and folded colony covered with a very fine, almost imperceptible aerial growth. Colony is at first pink, but becomes a deep, purplish-red to garnet in color. Reverse is very dark red. Colony throws sectors of white fluffy growth.
T. rosaceum	N. F. Conant, Duke University	359a	Isolated from white, fluffy sectors of No. 359, and obtained in single spore culture. Rapid-growing thick, fluffy colony. May develop a small amount of pink pigment on reverse.
T. megnini (strain Oscar/ Langeron)	R. Vanbreuseghem, Antwerp, Belgium	A-283	Colony almost flat with very shallow radial grooves. Surface white, may become slightly pink. Deep rose at border and on reverse.
T. megnini (strain Pollacci)	Central Bureau of Fungus Cultures, Netherlands	A-300	Colony is flat and covered with white fluffy surface growth. It may become pink on surface and red in center on reverse after 4 to 6 weeks.
T. vinosum	R. C. Artagaveytia-Allende No. 198 (Sabouraud's Collection, Paris, No. 193)	A-389	Colony flat with regular radial grooves. Surface downy, later becoming fluffy. At first cream to tan, later becomes deep rose both on front and reverse.

TABLE II

CHARACTERISTICS OF *Trichophyton gallinae* STRAINS ON SABOURAUD DEXTROSE AGAR

Culture identification as received	Source	CDC No.	Description of colony on Sabouraud dextrose agar after 4 weeks
A. gallinae	R. W. Benham No. 299.12 (Sabouraud's Collection, Paris)	A-7	Rapid-growing with irregular radial grooves, or irregularly folded over entire surface. White, downy to fluffy surface growth. Some light pink color develops in center or in a ring near periphery. Reverse of colony and surrounding media colored a strawberry pink which later becomes deep red.
A. gallinae	N. F. Conant No. 137 (Sabouraud's Collection, Paris)	A-25	Colony, rapid-growing, flat with faint suggestion of radial grooves. White downy to fluffy surface. No pigment.
T. megnini (syn.: *A. gallinae*)	American Type Culture Collection No. 7102	A-368	Similar to A-25.
T. megnini	A. Cury, Inst. Oswaldo Cruz, Brazil No. 2684 (Isol. from a cock, Dec. 1948)	A-397	Rapid growing, with central elevation and irregularly folded central area. Cracks appear on summits of folds. Surface white and fluffy. No pigment on reverse.
T. megnini	A. Cury, Inst. Oswaldo Cruz, Brazil No. 2806 (Isol. from a cock, Feb. 1950)	A-398	Slower growing than above, and almost flat with irregular radial grooves. Border irregularly fringed. Surface velvety, at first light pink, later a deep salmon. Pigment on reverse, strawberry pink at first, later becomes a deep red and spreads throughout entire medium.

inorganic nitrogen sources were added in amounts which contained equivalent weights of nitrogen (50 mg. N per 100 ml.). In some cases 0.2 or 0.5 percent of a vitamin-free, acid-digested casein (Baltimore Biological Co.) [2] was substituted for the inorganic nitrogen. The media were autoclaved for 10 min. at 120° C. Buffered aqueous amino acid solutions were prepared, sterilized by autoclaving (or by filtration, if glutamine was present) and added singly and in combinations, in amounts containing equivalent weights of nitrogen, to melted partially cooled (approx. 45° C.) basal agar. The water-soluble vitamins were similarly prepared in buffered distilled water. The fat-soluble vitamins were dissolved in acetone. All of the vitamin solutions were sterilized by filtration and added to the melted, partially cooled basal agar. The glassware was acid-cleaned, and chemicals were of C. P. quality.

RESULTS

I. *Trichophyton megnini*

A. *Utilization of nitrogen sources:* All strains which had been labelled *T. rosaceum, T. megnini* or *T. vinosum* when received for study (with the exception of strain *A-368* obtained from the American Type Culture Collection, which was listed as "*T. megnini,* syn. *A. gallinae*") reacted similarly in that they required organic nitrogen to grow. No growth whatsoever of these strains (Nos. *424, 425, 359, 359a, A-283, A-299, A-300,* and *A-389*) was obtained on basal medium containing NH_4NO_3, NH_4Cl, or $NaNO_3$ (50 mg. N per 100 ml., or 250 mg. N per 100 ml.) as a sole nitrogen source. Substitution of 0.2 percent of a vitamin-free, acid-digested casein allowed a small amount of growth with all strains, and heavy growth was obtained when larger amounts of casein (0.5 and 1.5 per cent) were added. It appeared that these strains, all morphologically typical of *T. megnini,* were self-sufficient for the vitamins, but required some amino acid or combination of amino acids present in casein in order to grow.

Various mixtures of the following amino acids were prepared:

[2] The company name is used as a means of identifying products under discussion and does not represent endorsement of the products by the Public Health Service.

glycine, dl-alanine, b-alanine, dl-serine, dl-threonine, dl-valine, l-leucine, dl-isoleucine, dl-norleucine, l-proline, l-tryptophane, l-tyrosine, dl-phenylalanine, l-lysine, l-arginine, l-histidine, dl-ornithine, l-cystine, l-cysteine, dl-methionine, l-aspartic acid, l-glutamic acid, l-asparagine, l-glutamine, l-hydroxyproline. Each mixture was so adjusted that when it was added to the basal medium the total nitrogen content of the medium was 50 mg. per 100 ml. Amino mixture I contained all the above amino acids. In amino mixture II, l-hydroxyproline was omitted, as this amino acid has been shown to have inhibitory effects on the growth of various dermatophytes (**19**). Succeeding amino acid mixtures were prepared, each with a different group omitted. These groups, as well as the single amino acids, were prepared in solutions for separate testing.

When growth with amino mixture I was compared with that with amino mixture II, it was apparent that hydroxyproline has a slight inhibitory effect on the growth of all the strains studied. This is similar to the findings of Robbins and McVeigh (**19**) for other species of dermatophytes.

Further testing with the various amino acid mixtures and single amino acids showed that growth of the *T. megnini* strains occurred only when l-histidine was present. Thus these strains were shown to have a requirement for this single amino acid similar to that of the mutant *Trichophyton violaceum* strain previously reported (**9**). These are the only dermatophyte strains which have been reported to require a specific amino acid. Silva [3] has found a similar requirement for l-histidine in a group of strains of *T. megnini* which she is studying.

The minimum effective concentration of l-histidine was determined by adding graded amounts of this amino acid to a series of tubes each containing 5 ml. NH_4NO_3 basal broth (50 mg. N per 100 ml.), and inoculating with small fragments of the culture grown on 0.2 percent casein agar. The tubes were incubated at 25° C. for 4 weeks. Four strains (*424, A-302, 359,* and *359a*) were titrated. The smallest amount of l-histidine which allowed growth in 5 ml. NH_4NO_3 broth was 2.4–4.8 μg. for all strains tested. No growth was obtained in tubes containing less than 2.4 μg. histidine.

[3] Personal communication.

Maximum growth occurred in tubes containing 75–200 μg. Definite inhibition of growth was observed in tubes containing doses larger than 5.0 mg., and complete inhibition occurred in the presence of 10.0 mg. histidine. The titration with histidine was repeated with these same strains in a basal broth containing a mixture of all other amino acids except hydroxyproline, and adjusted so that the medium again contained 50 mg. N per ml. before additions of the histidine. In such a medium, the minimum effective concentration of histidine was 300 μg. or approximately 100 times the amount required to initiate growth in an NH_4NO_3 medium of similar N content. Maximum growth in the amino acid medium was obtained in tubes containing 500 to 1000 μg. histidine. Some inhibition occurred in the presence of 10 mg. or more.

Although a smaller amount of histidine was required to initiate growth in inorganic nitrogen media than in the organic nitrogen media, the growth of these strains on NH_4NO_3-histidine medium was scant and of poor quality. On the other hand, the addition of a mixture of amino acids (all except hydroxyproline) to a medium adequately supplied with histidine allowed good growth of all strains. When the amino acids were tested separately with histidine, the greatest stimulatory effects were produced by asparagine, glutamine, and arginine. The combination of any of these three amino acids with histidine provided a medium on which growth was comparable to that on 0.5 percent casein agar. The typical pigment was produced and microconidia were found in the aerial mycelium. Other amino acids which showed some slight stimulatory effect in combination with histidine were alpha alanine, beta alanine, phenylalanine, glycine, leucine, tyrosine, and proline.

B. *Effect of vitamins:* Mixtures of water-soluble and fat-soluble vitamins were added to the basal agar in an effort to determine whether they could replace histidine, or whether they stimulated growth further on a histidine-containing medium. All the vitamins were tested singly and in combination in an NH_4NO_3 basal agar and on 0.2 and 0.5 percent casein agars. The vitamins were used in final concentration per ml. as follows: B_{12}, 0.15 μg.; biotin, 0.002 μg.; thiamin, 0.2 μg.; riboflavin, 1.0 μg.; ascorbic acid, 0.2 μg.; choline, 5.0 μg.; folic acid, 5.0 μg.; i-inositol, 50.0 μg.; nicotinic acid, 1.0 μg.; nicotinic amide, 1.0 μg.; para-aminobenzoic acid,

5.0 μg.; Ca pantothenate, 0.2 μg.; pyridoxine, 0.2 μg. A complete mixture was prepared, as were 13 mixtures with one vitamin omitted from each. None of the single vitamins or mixtures was found to substitute for histidine, nor did they have a stimulatory effect on the growth of the fungus when added to casein-containing media (FIG. 1).

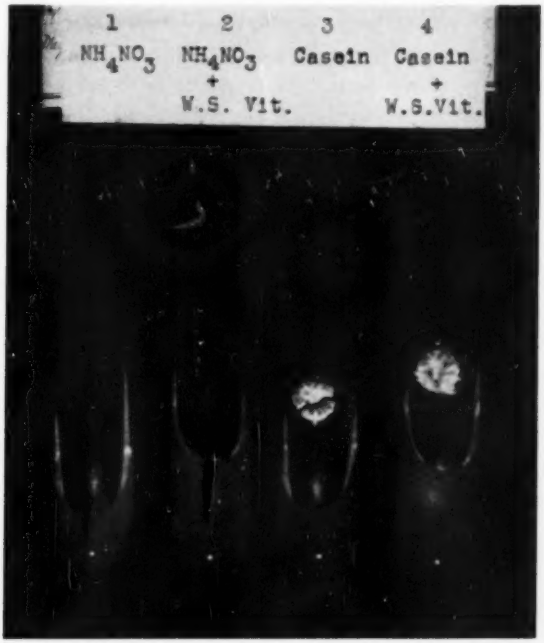

FIG. 1. *T. megnini*. Nutrition tubes showing no growth on NH_4NO_3 dextrose agar, but good growth on casein dextrose agar. The addition of a mixture of water-soluble vitamins has no effect with either medium.

Similarly, mixtures of the fat-soluble vitamins dissolved in acetone were tested. These substances were used in final concentration per ml. as follows: Vit. D_2 (calciferol) 5.0 μg.; Vit. A (beta-carotene) 2 mg.; Vit. K_3 (menadione, 2-methyl-1,4-napthoquinone) 0.5 μg.; Vit. E (alpha tocopherol acetate) 0.5 μg. The various increments of the fat-soluble vitamin solutions were brought to equal volumes (10 ml.) with acetone before being added to 100 ml. of the basal medium, 10 ml. of acetone being added to 100 ml. of

the basal medium for control tubes. The acetone was found to have no inhibitory effect on the growth of any of the strains. None of the fat-soluble vitamins or mixtures of these vitamins could substitute for histidine nor did they show any stimulatory effect on the growth of these strains on a 0.5 percent casein agar. However, some inhibitory effect was observed with the complete mixture, and tests with the single vitamins revealed that this was due to vitamin K_3. As more concentrated solutions (10 × and 50 × the original dosage) of this vitamin were tested, increased inhibition of growth was obtained. Three of the strains were completely inhibited by the addition of 250 μg. vitamin K per 5 ml. casein agar. Inhibition of growth of the dermatophytes by various K vitamins has been reported by Arêa Leão and da Rocha Furtado (1).

C. *Morphological studies:* A description of the cultures of the *T. megnini* strains grown on Sabouraud dextrose agar (Difco) is given in TABLE I. FIG. 2 shows the range of colony variation of these strains. Strains *424, 425, A-299, A-389,* and *A-302* produced very similar colonies (examples, FIG. 2a and b) which correspond closely to the description of the species given by Sabouraud. Microscopic examination of these revealed a fine mycelium and, in the aerial growth, most of the strains showed a few delicate microconidia scattered along the hyphae. Strain *A-283* produced a more rapidly-growing flat colony with less tendency to form radial grooves. The aerial mycelium was more abundant, giving the colony a fluffy appearance (FIG. 2c). Strain *A-300* was completely flat and was covered with an abundant fluffy surface growth (FIG. 2d). These last two colonies were white up to 3 to 4 weeks, when they showed a small amount of pink pigment on the surface and deep rose in the center on the reverse side of the colonies. Microscopically they showed the thin, delicate mycelium associated with pleomorphism, and only a few microconidia were seen. Strain *359* (FIG. 2e) presented quite a different colonial appearance from the above. It was slow-growing and developed as a small, heaped, highly folded colony. The surface was covered with a very fine, short velvet. At first the colony was pinkish, but later it became a deep garnet color. No microconidia were found. In several transplants there occurred sectors of white fluffy growth which grossly

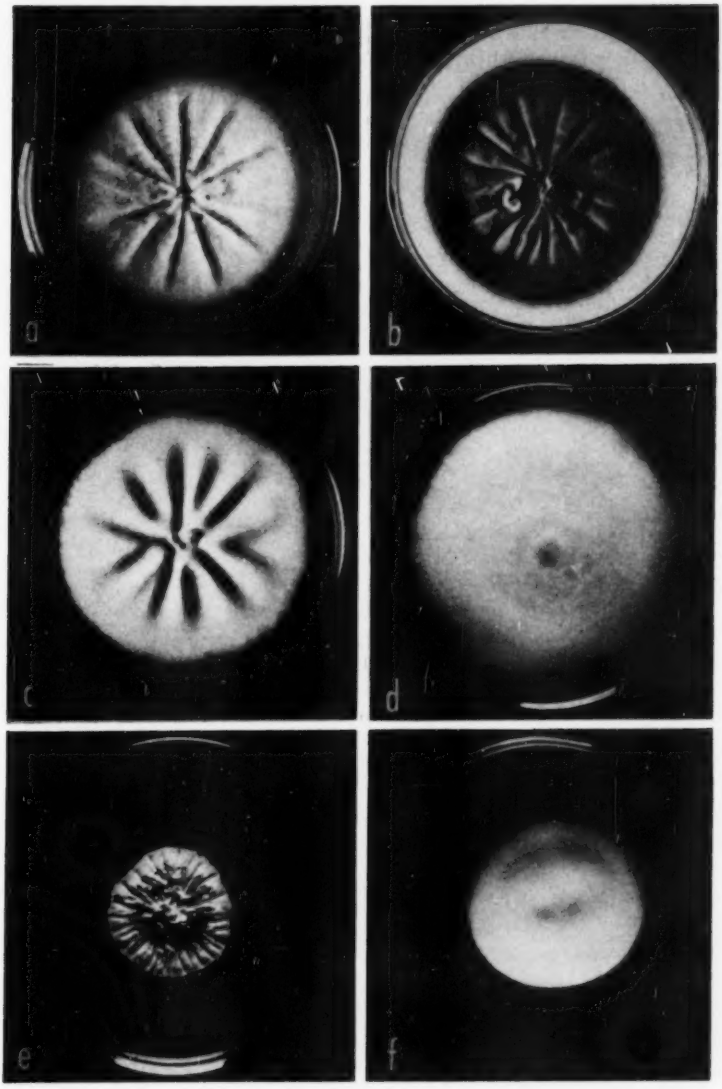

FIG. 2. *T. megnini.* Colonies on Sabouraud dextrose agar: a. *424*, b. *A-302*, c. *A-283*, d. *A-300*, e. *359*, f. *359a*.

appeared to be pleomorphic. However, in such sectors the mycelium was of normal diameter and was covered with many microconidia. A spore suspension was obtained from this growth, and

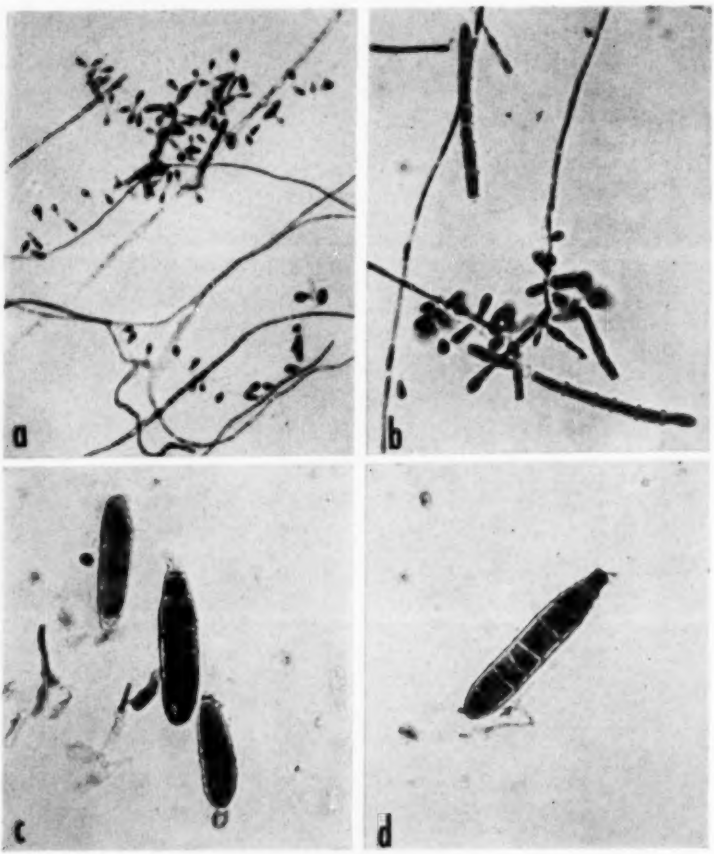

FIG. 3. a. *T. megnini*, microconidia; b. *T. megnini*, macroconidia; c and d. *T. gallinae*, macroconidia. (All photographs at same magnification.)

a single spore strain, *359a* (FIG. 2f), was secured according to the method described by Georg (**9**). Some transplants of this strain eventually showed some rose pigment, but they never reverted to the heaped, folded, deep red growth of the mother culture.

Since all of these strains had been shown to be deficient for histidine and were stimulated by the presence of other amino acids, it was of interest to determine whether substitution of a nitrogen source such as trypticase (an enzymatic digest of casein), known to be rich in amino acids, for the neo-peptone of Sabouraud dextrose agar would have any effect on the growth or spore production of these strains. A trypticase dextrose agar similar to the Sabouraud dextrose agar was prepared with the substitution of trypticase (Baltimore Biological Co.) for the neo-peptone. All of the strains grew more rapidly on this medium, and the colonies were heaped and folded in the center and showed many fine radial grooves. The aerial growth was increased, and microscopic examination revealed microconidia in all strains (FIG. 3a). Strains which had produced few microconidia on Sabouraud dextrose agar produced them much more abundantly on this medium, both along the sides of the hyphae and in terminal clusters. Several strains (*424, 359a,* and *A-399*) produced what appeared to be delicate macroconidia. These latter structures, composed of 2 to 5 cells, were more or less club-shaped in form. They developed both terminally and from the sides of the mycelium. They were thin-walled and only slightly wider than the vegetative mycelium which bore them, but were easily distinguished because they were filled with dense protoplasm which stained deeply. They varied considerably in length from a short two-celled structure, 10 mμ long, to a few which reached 35 mμ in length (FIG. 3b).

Sabouraud described both types of conidia in 1909 (**21**). He had observed microconidia along the sides of the hyphae ("en thyrse") and states that Truffi in 1902 had seen them in clusters ("en grappe"). In hanging drop cultures, Sabouraud saw pleuricellular structures in spindle shape ("fuseaux") or in the shape of the clapper of a bell. These were intercalary or at the extremities of the mycelial filaments and appeared as slightly thickened, multicellular areas of dense protoplasm. He stated that they resembled structures common to most of the trichophytons. Clear drawings were presented of the mycelium, microconidia, and "terminal fuseaux."

II. *Trichophyton gallinae*

A. *Utilization of nitrogen sources:* Five of the strains studied (Nos. *A-7, A-25, A-368, A-397,* and *A-398*) grew well on NH_4NO_3 agar, and their growth was not stimulated by additions of the various amino acids or casein.

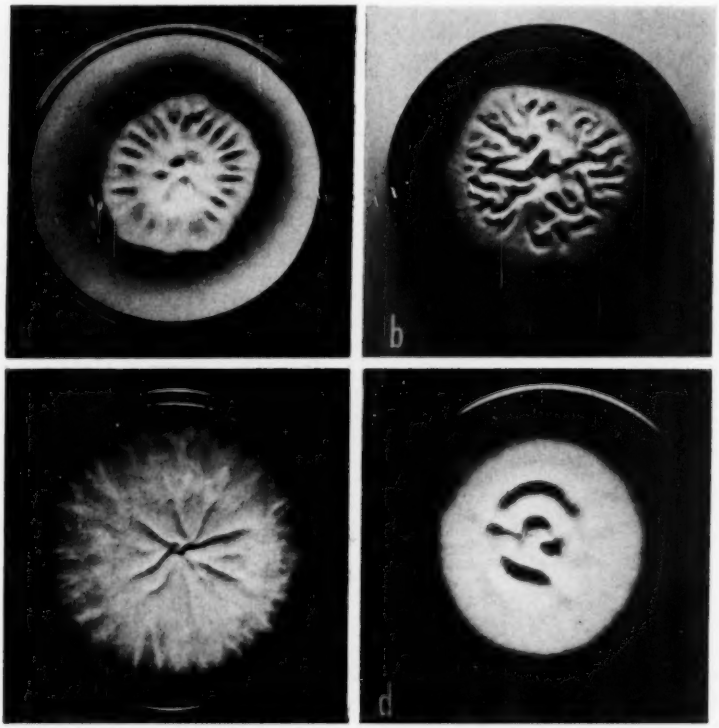

FIG. 4. *T. gallinae.* Colonies on Sabouraud dextrose agar: a. *A-7* (young colony with halo of pigment), b. *A-7* (older colony with entire medium pigmented), c. *A-398*, d. *A-397*.

B. *Effect of vitamins:* No stimulation of growth was obtained by the addition of complete mixtures of either the water-soluble or fat-soluble vitamins.

C. *Morphological studies:* Description of the growth of these strains on Sabouraud's dextrose agar is given in TABLE II. Two

of the strains, *A-7* and *A-398*, produced characteristic white, irregularly folded colonies which later developed a faint pink to rose color on their surfaces and a soluble strawberry-red pigment which diffused throughout the medium. Old colonies developed cracks on the summits of the folds, and the entire agar medium in the tube or plate became a very deep red. These colonies appeared to be typical of *T. gallinae* as described by Sabouraud (**22**). The other strains in this group were similar except that they were less folded and did not produce pigment. Since they had been originally identified as either "*A. gallinae*" or "*T. megnini*," it is assumed that they must have shown some red pigment at one time. The loss of pigment in stock culture is not uncommon among the dermatophytes. FIG. 4a shows a young colony of *T. gallinae*, strain *A-7*, surrounded by a halo of pigment. FIG. 4b represents an older colony of strain *A-7* which is irregularly folded over the surface, and where the entire medium is colored by the deep strawberry-red pigment. FIG. 4c and d illustrate two other colonial variations of *T. gallinae*. 4c (*A-398*) has a pink velvety surface, and the entire medium is colored a deep red; 4d (*A-397*) has a white fluffy surface, and has lost the ability to produce pigment.

Microscopic examination of strains *A-7*, *A-25*, *A-368*, and *A-397* revealed only a thin mycelium and numerous chlamydospores. Production of reproductive spores could not be induced by the use of special media, and it seems probable that these cultures which have been on artificial media for several years have become largely pleomorphic. One of the more recently isolated cultures, however, No. *A-398* (isolated from a cock in Brazil, 1950), produced both microconidia and macroconidia. The microconidia were few in number and appeared as small pyriform to elongate structures scattered along the mycelium. They appeared similar to the microconidia of *T. megnini*. The macroconidia, on the other hand, were present in relatively large numbers. These structures were larger, especially in width, and were more distinctly clavate than those of *T. megnini*. They varied from a short 2-celled spore, 15 mμ long, to ones with 9 to 10 cells, 50 mμ in length. The average width was 6 to 8 mμ. The walls were smooth and very dis-

tinct (FIG. 3c and d). These structures resemble those described by Sabouraud (22) and Baudet (2) for *T. gallinae*. A comparison of the macroconidia of *T. megnini* and *T. gallinae* as seen in camera lucida drawings from the strains studied, FIG. 5A and B,

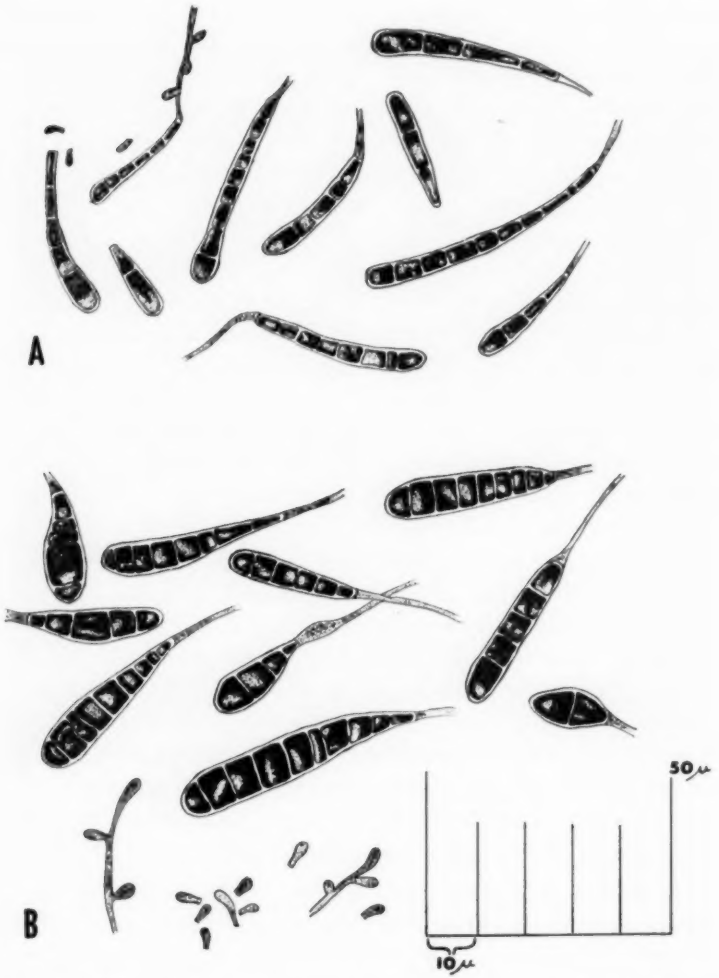

FIG. 5. Microconidia and macroconidia. A. *T. megnini*. B. *T. gallinae* (camera lucida).

TABLE III

COMPARISON OF *Trichophyton megnini* AND *Trichophyton gallinae*

	T. megnini	*T. gallinae*
Colony on Sab. dex. agar	Slow growing, flat, usually with regular radial grooves. Surface velvety, at first white, then pink, and later a deep rose. Edge of colony smooth, but often scalloped. Reverse of colony at first rose, and later a deep red. Pigment does not extend beyond colony.	More rapid-growing, at first flat, but develops irregular folds. In old colonies cracks may appear on summits of folds. Surface downy to fluffy; usually white, but may develop pink areas or may be all a light pink. Edge of colony tends to be irregular, sometimes fringed. Reverse of colony a light strawberry-red, and pigment diffuses out into the medium. In an old culture, entire medium is a deep red.
Growth on NH_4NO_3 medium	No growth	Good growth
Special Nutritional Requirements	Requires l-histidine. Growth is stimulated by the presence of other amino acids.	No special requirements. Growth is not stimulated by a mixture of amino acids or casein.
Microscopic Morphology	Production of reproductive spores is stimulated by a medium rich in amino acids.	Production of reproductive spores is not stimulated by special media.
(a) Microconidia	May be numerous, small, pyriform to elongate. Occur along mycelium or in clusters.	Usually few in number, but similar to those of *T. megnini*.
(b) Macroconidia	Rare, very slightly clavate, 2–8-celled, 10–35/3–6 mμ thin, smooth walls.	May be numerous, distinctly clavate, 2–10 cells, 15–50/6–8 mμ. Smooth walls, distinct and slightly thicker than in *T. megnini*.

clearly shows that on the basis of these structures, the two species may be differentiated.

TABLE III summarizes the physiological and morphological characteristics of *T. megnini* and *T. gallinae*.

SUMMARY

1. A review of the history and classification of *Trichophyton megnini* and *Trichophyton gallinae* is given.

2. Nutritional and morphological studies of strains available further emphasize the differences between these two species which have so often been confused in the past.

3. All of the *T. megnini* strains were shown to require the addition of the amino acid l-histidine in order to grow on an ammonium nitrate-dextrose medium. L-histidine could not be replaced by any other amino acid or combination of amino acids. In the presence of histidine, the amino acids which provided greatest stimulation were: asparagine, glutamine, and arginine. All strains were somewhat inhibited by hydroxyproline. The minimum effective concentration of histidine was determined in NH_4NO_3 broth, and also in an amino acid mixture complete except for hydroxyproline and histidine.

4. No stimulation of the growth of the *T. megnini* strains was observed upon the addition of water-soluble or fat-soluble vitamins to a histidine medium. Vitamin K_3 had an inhibitory effect.

5. Spore production of most of the *T. megnini* strains was stimulated by growth on media rich in amino acids, such as trypticase-dextrose agar. Further addition of histidine to this medium did not increase growth or spore production. The microconidia and macroconidia of *T. megnini* are described.

6. Five strains studied showed morphological characteristics suggestive of *T. gallinae*. These strains grew well on inorganic nitrogen medium and were not stimulated by the addition of casein or amino acids.

7. No stimulation of the *T. gallinae* strains was observed upon addition of water-soluble or fat-soluble vitamins.

8. One recently isolated strain showed microconidia and macroconidia characteristic of *T. gallinae*.

9. The physiologic and morphologic characteristics of *T. megnini* and *T. gallinae* are summarized.

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STUDIES OF AN INSECT MYCOSIS. IV. THE PHYSIOLOGY OF THE HOST-PARASITE RELATIONSHIP OF PLATYSAMIA CECROPIA AND ASPERGILLUS FLAVUS[1,2]

ALFRED S. SUSSMAN

(WITH 5 FIGURES)

Virulence does not refer to some "unique attribute of the microorganism," but rather "to the disease and to the host-parasite relationship" (Dubos **1945**). Because of the complexity of such disease relationships, quantitative measurements of virulence are difficult to make and even more difficult to interpret. However, since the infection of the cecropia moth, *Platysamia cecropia* L., by *Aspergillus flavus* Link has been shown to be amenable to physiological investigation (Sussman **1951a**, **b**, **1952a**), it was felt that a quantitative expression of virulence might be evolved through a study of the physiological aspects of this disease.

RESPIRATORY CHANGES IN DISEASED ANIMALS

The respiratory changes in diseased animals were first investigated. Diapausing pupae of *Platysamia cecropia*, obtained from Dr. C. M. Williams, were used in these experiments. The oxygen uptake of the intact animals was measured by the use of Scholander-Edwards microrespirometers immersed in a constant temperature water bath kept at 24° C. ± 0.5° C. and equilibrated for use as described previously (Sussman **1952**).

[1] Portion of thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Harvard University.

[2] The author would like to express his deepest appreciation and thanks to Professor William H. Weston, Jr., for his stimulation and guidance during these investigations. I should also like to thank Professors Kenneth V. Thimann and Carroll M. Williams for the continued technical help and fruitful discussion with which they eased the course of this work. Finally, Professor David R. Goddard kindly read part of this manuscript and offered many pertinent and penetrating suggestions.

Infection was accomplished by injecting 0.05 ml. of a heavy aqueous suspension of conidia of *Aspergillus flavus* directly into the animal's body cavity with a sterile hypodermic syringe and a needle with a diameter of approximately 0.017 cm. In order to minimize loss of blood due to the animal's movement, the needle was inserted laterally into the thoracic intersegmental membrane and was withdrawn very carefully, after injection of the inoculum. Control animals were injected with 0.05 ml. of sterile distilled water. Before injection, all animals were swabbed with 70% alcohol to prevent chance contamination.

Unless otherwise stated, the treated animals were held in an incubator kept at 25° C. and were removed only during the time that measurements were made.

Standard deviations and statistical testing of the significance of the results were done by the group comparison methods of Snedecor (**1946**).

RESPIRATORY CHANGES IN PARASITISM

The respiration of diseased animals was first investigated. Previously chilled diapausing pupae were infected and their oxygen uptake measured daily for a two-week period. (The oxygen uptake of control animals, and other aspects of their respiratory metabolism were reported in a previous paper (Sussman **1952**) so that only brief mention of these results, for comparative purposes, will be made later.)

It is at once obvious from the results in FIG. 1 that an enormous increase in oxygen uptake occurs in the infected animal. This increase is maintained even when shrinkage and blackening of the integument would indicate that the animal had died. After the maximum is reached, the oxygen uptake of the animal declines very slowly until the time when the animal is almost completely dehydrated.

In order to determine what proportion of this increase could be attributed to changes in the metabolism of the host, it was necessary to measure the extent of the parasite's oxygen uptake. With this in mind, several pupae were inactivated by immersion for 30 minutes in water heated to 80° C. Since, upon checking the respiration of these animals, it was found that they showed no appreciable

oxygen uptake, it was felt that the enzymes involved in respiration had been inactivated by the heat treatment. That tyrosinase, at least, was inactivated was proven by the fact that blackening of these animals never occurred, even though they were heavily infected with the fungus. Therefore, animals treated in this way were inoculated and the changes in oxygen uptake measured. Consequently, it was now possible to estimate the extent of the change in the respiration of the host due to parasitism inasmuch

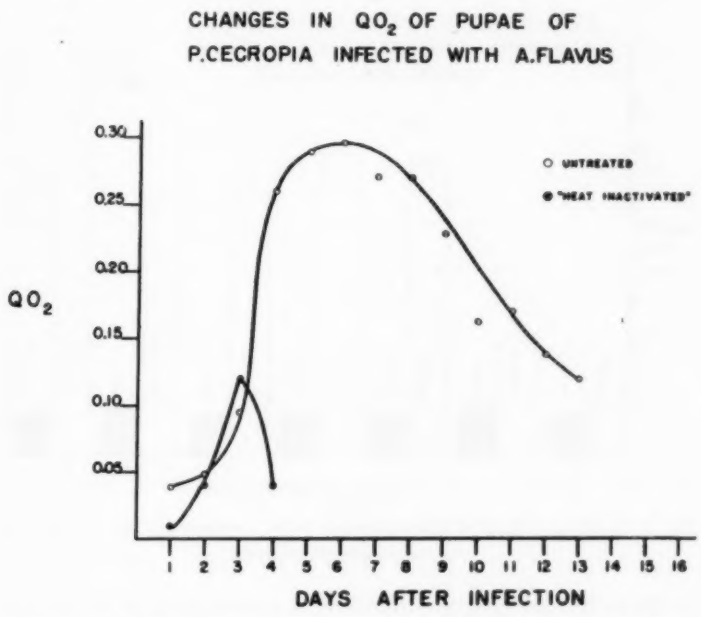

FIG. 1. Changes in Q_{O_2} of pupae of *P. cecropia* infected with *A. flavus*.

as the oxygen uptake of the heated, inoculated pupae could be assumed to be that of the parasite alone. The results are plotted in FIG. 1, and it can be seen that there is about a ten-fold rise in the oxygen uptake of the host, induced as a result of parasitism.

WEIGHT CHANGES IN PARASITISM

The previous results, plus the observation that infected pupae soon shrivel up and shrink in size, suggested that drastic changes

in the weight of the animal occur as a result of parasitism. Therefore, the weight changes were next investigated.

The first series of experiments were designed to determine the difference in the amount of weight lost by diseased and by healthy animals. Animals were infected in the manner previously described and were then weighed daily and the loss of weight, in milligrams, recorded. These figures were then compared with those obtained for uninoculated controls and the results plotted in FIG. 2.

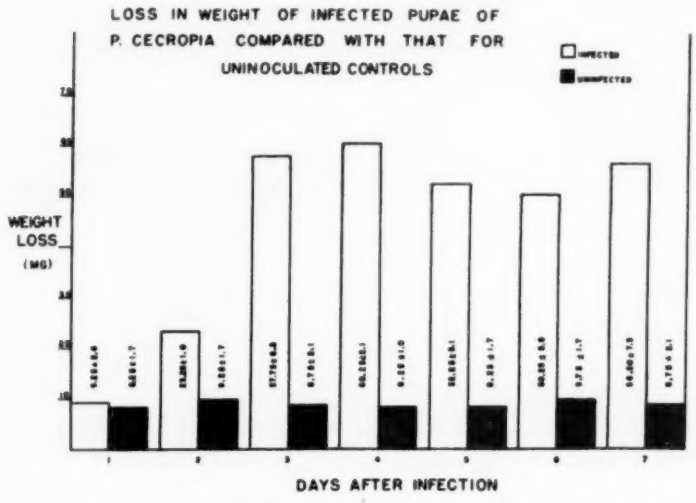

FIG. 2. Loss in weight of infected pupae of *P. cecropia* compared with that for uninoculated controls.

These results indicate that about a 7-fold increase in the rate at which weight is lost occurs within two days after infection. Moreover, this increase is maintained for at least 6 days after infection after which the rate decreases steadily. It is of interest to note that blackening and shrinking of the animal begins three days after infection at which time the rate of weight loss is still at its maximum. Furthermore, FIG. 3 shows that the curve for weight loss is almost parallel with that for the respiratory activity of diseased animals, as determined in the preceding experiments, except that the rate at which weight is lost reaches a maximum sooner than does the oxygen uptake.

Having found that increased weight loss results from the attack of *A. flavus* on the pupae, it now became of interest to investigate the route by which such water was lost in the pupae. To investigate this problem, the spiracles and the anal orifice of infected animals were coated with a waterproof resin which has been found to be a harmless, yet effective, seal for insect spiracles (Ramsey **1948**).[3]

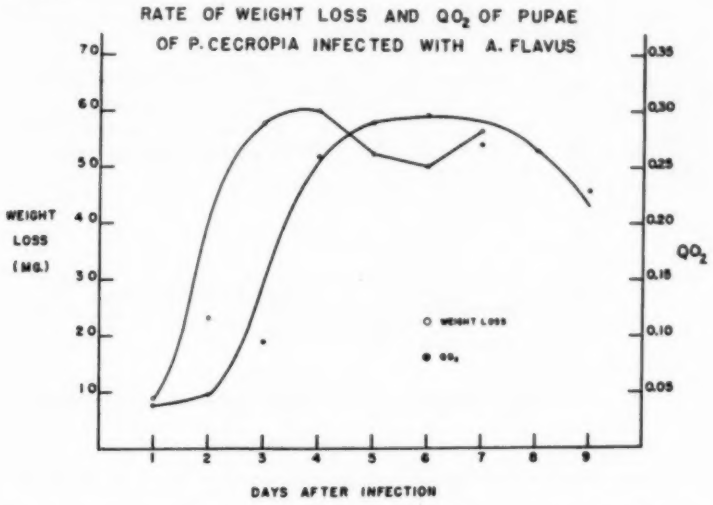

FIG. 3. Rate of weight loss and Q_{O_2} of pupae of *P. cecropia* infected with *A. flavus*.

The following treatments were given:

Set No. 1. Injected with 0.05 ml. of sterile distilled water; spiracles sealed with resin.

Set No. 2. Injected with 0.05 ml. of a spore suspension of *A. flavus;* spiracles not sealed with resin.

Set No. 3. Injected with 0.05 ml. of sterile distilled water; spiracles not sealed with resin.

Set No. 4. Injected with 0.05 ml. of a spore suspension of *A. flavus;* spiracles sealed with resin.

[3] While the exact nature of this substance is not known, it is produced by the Southern Adhesives Corporation and is designated as compound No. 115-20.

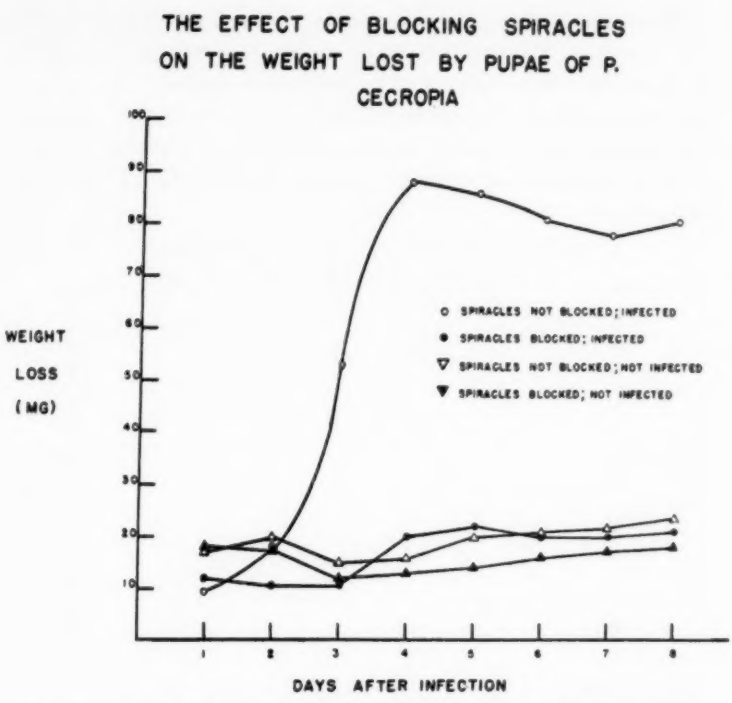

FIG. 4. The effect of blocking spiracles on the weight lost by pupae of *P. cecropia*.

The animals were then incubated at room temperature (28° C.) and weighed daily. The results of this experiment are given in FIG. 4 and TABLE I.

These results indicate that the major portion of the weight lost during infection is lost through the spiracles. Sealing of the spira-

TABLE I

COMPARISON OF THE AMOUNT OF WEIGHT LOST PER DAY IN PUPAE OF *P. cecropia* TREATED AS DESCRIBED IN TEXT. DATA COMPUTED FROM RESULTS OBTAINED FROM THE 4TH THROUGH THE 8TH DAY AFTER ORIGINAL TREATMENT. FIVE ANIMALS WERE USED IN EACH TREATMENT.

Treatment	Weight loss (mg.) Infected	Non-infected
Sealed	20.6 ± 1.0	16.6 ± 2.2
Unsealed	82.8 ± 4.1	20.6 ± 3.0

cles has no significant effect upon the amount of weight lost by the uninfected animals, as shown by a Chi-squared test, so it is probable that such loss in weight must be attributed to factors other than evaporation from the spiracles.

EFFECT OF TEMPERATURE ON WEIGHT LOSS

For this purpose, animals were inoculated as before and were incubated at several different temperatures including 28° C., 20° C., 15° C., 10° C., and 5° C. The atmosphere of the incubators and cold rooms in which these animals were held was kept at approximately 75% relative humidity. Controls were held at the same temperatures but were injected with 0.1 mm. of sterile distilled water instead of the spore suspension. All animals were removed

TABLE II

EFFECT OF TEMPERATURE UPON THE TIME NECESSARY FOR SYMPTOMS OF DISEASE TO APPEAR IN PUPAE OF *P. cecropia* INFECTED WITH *A. flavus*

	Days after infection	
Incubation temperature	Movement of abdomen ceased	Blackening of blood and shriveling of integument visible
28° C.	2	3
20° C.	3	7
15° C.	7	13
10° C.	normal at end of 60 days	normal at end of 60 days
5° C.	normal at end of 60 days	normal at end of 60 days

once daily for weighing but were otherwise maintained continually at their respective constant temperatures. At the time of weighing, the animals were inspected for changes in the color of the blood and for visible signs of dehydration, such as shriveling of the integument. At the same time, the animal's response to stimuli was tested by noting whether the abdomen moved when slight pressure was applied to the thorax.

In these experiments, it was assumed that death of the pupae occurred upon the blackening of their blood and upon their failure to respond to stimuli. Consequently, the data in TABLE II indicate that the minimum temperature at which the disease can kill the insect lies between 10° C. and 15° C.

Using the above data, then, as a basis for comparison, we see

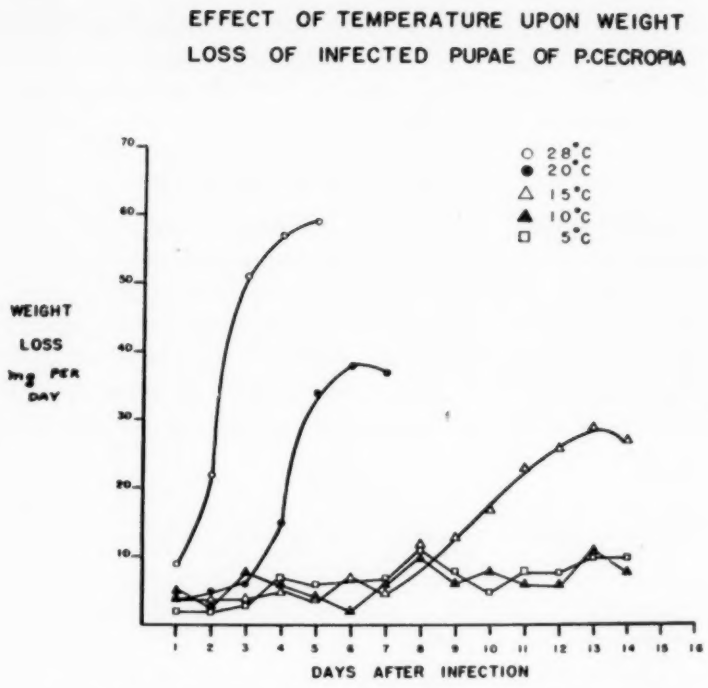

FIG. 5. Effect of temperature upon weight loss of infected pupae of *P. cecropia.*

that the results obtained by plotting weight loss against time (FIG. 2) show that death occurs, in animals held at 28° C., 20° C., and 15° C., at the time when the rate at which weight is lost is at its maximum. Animals held at these temperatures undergo a rapid increase in weight loss as a result of parasitism, this weight loss being directly proportional to the temperature. On the other hand, animals held at 10° C. and 5° C. failed to show any significant increase in the normal rate of weight loss and, as is seen in TABLE II, showed no other symptoms of infection. The data in FIG. 5, then, demonstrate quantitative differences in the effect of temperature on weight loss.

In order to learn whether the failure of the symptoms of disease to appear in infected animals held at the low temperatures was

due to increased resistance of the host, or merely to an inhibition of the fungus by low temperatures, these animals were returned to room temperature and observed for symptoms of the disease. Typical symptoms of death appeared within 3 days after their removal from the cold rooms so that it was apparent that the fungus was not destroyed by the insect during the incubation at low temperatures. This suggests that phagocytosis and other defense mechanisms of the insect did not serve to prevent the infection, but rather that cold temperatures inhibited the growth of the fungus.

DISCUSSION AND CONCLUSIONS

Although the results of the experiments on the respiration of parasitized animals suggest that there is about a ten-fold increase in the oxygen uptake of the host, it cannot be claimed that this figure is more than an approximation. This figure is much higher than the 250% increase shown to occur in mildew-infected wheat (Allen and Goddard **1938**) but the materials are so different that it is difficult to compare these experiments with the current ones. In determining the magnitude of the parasite's oxygen uptake it was necessary to assume that the heat-treated animal provided conditions for the growth of the fungus which were equivalent to those in the normal animal. That this is probably not so is indicated by the fact that the rise in the Q_{O_2} of the parasitized "heat-treated" pupae is much more sudden than that for the untreated parasitized animals. It is possible that heating the animals inactivated certain of the mechanisms by which the normal animal delays or prevents development of invading pathogens, so that the development of the fungus is much more rapid in the treated animals. In any case, it is probably safe to assume that the magnitude of the pathogen's respiration is equal in both cases, but that the rate at which it develops is definitely enhanced in the heat-treated animals. It is possible that differential poisoning experiments and measurements of CO_2 output will help clarify the nature of this respiratory increase.

Roughly paralleling the rise in oxygen uptake was a similar increase in the loss of weight of infected pupae. That most of this loss is due to the evaporation of water is suggested by the

work of Agrell (**1947**) who analyzed some of the factors involved in the weight loss of pupae of *Phalera bucephala* L. (Lep.). According to this investigator's calculations, the RQ of the animal determines whether the respiratory exchange will represent a gain or a loss in weight; only at an RQ of 0.727 will there be no gain or loss. This figure is derived from the ratio of the molecular weights of O_2 and CO_2 so that only when 44 moles of O_2 are respired and 32 moles of CO_2 given off will there be a constancy in the weight of the pupa. Since diapausing pupa of *P. cecropia* have been shown to have an RQ of 0.65–0.75 (unpublished experiments of the author), it becomes clear that any weight loss occasioned by the respiratory exchange is negligible compared with the water loss. This conclusion is substantiated by the work of others who have found that respiratory metabolism during pupal development is characterized by a low RQ (cf. Taylor and Steinbach **1931**; Ashbel **1931**). In these cases there would actually be a small gain in weight to counteract the loss due to the evaporation of water.

The site of water loss in the healthy pupa appears to be the integument, inasmuch as sealing the spiracles of such animals had little or no effect on the amount of water lost. On the other hand, the route of water loss for diseased animals appears to be by way of the spiracles. This suggests that one of the ways by which the parasite destroys the insect is by interfering with the functioning of the animal's tracheoles and spiracles either directly, or indirectly, by destroying the animal's nervous system. That this is probably the case is confirmed by the histopathological evidence presented in a previous paper (Sussman **1952a**), wherein it is shown that tracheoles and ganglionic tissue are indeed attacked and destroyed by the fungus.

The results of the experiments on the effect of temperature on the disease indicate that the rate at which weight is lost varies directly with the temperature. Also indicated is the fact that the symptoms of death appear most quickly at the higher temperatures. In general, comparing these results with those obtained for other *Aspergillus* infections of insects, we find rather good agreement. For example, Boyce and Fawcett (**1947**) found that mealybugs

were killed by a fungus related to *A. parasiticus* at the end of 36 hours at 31° C., but that twice this amount of time was required to kill them at 21° C. Similarly, Blochwitz (**1929**) states that *Aspergillus* infections of *Drosophila* killed the animal most quickly at 35° C. but required progressively longer periods of time to kill them at temperatures below 25° C. On the other hand, Lepesme (**1939**) showed that *A. flavus* would not kill *Schistocerca gregaria* at a temperature below 18° C., so it is probable that the minimum temperature required for a parasite to kill an insect varies with the strain of parasite, and/or the strain of the animal parasitized.

That low temperatures do not enhance the resistance of the host but rather inhibit the growth of the fungus is demonstrated by the preceding experiments. These data are supported by some unpublished experiments of Dr. Ralph Emerson, of the University of California, in which he found that the minimum temperature for the growth of *A. flavus* is about 13° C.

There are other reasons why these experiments on the relation of temperature to weight loss in the diseased insect must be interpreted with caution since so many facets of the host-parasite relation are affected by even small changes in temperature. For example, Agrell (**1947**) found that the RQ, as well as the weight loss of pupae of *P. bucephala*, was changed upon the alteration of temperature. Since changes in RQ affect the proportions of the gases exchanged during respiration (cf. above), it is possible that factors other than water loss enter into the calculations of weight loss.

Moreover, the relative humidity of the environment plays an important part in determining the quantity of water lost by evaporation from the insect (Mellanby **1935**). That this factor must be considered in the case of insect diseases is shown by the work of Wallengren and Johansson (**1929**) who found that larvae of *Pyrausta nubilalis* Hb., infected with *Metarrhizium anisopliae*, endured the mycosis approximately twice as long at a humidity of 60–70% than at saturated humidity. A similar effect was noted by Lepesme (**1939**) while working on an infection of *Schistocerca gregaria* Forsk. caused by *A. flavus*. Since no significant differences in the rate at which water was lost in the control animals

could be detected, it was concluded that this variable was controlled in the present experiments.

Finally, consideration must be made of the influence of temperature on the fungus itself. In fact, the steeply rising portion of the curves for weight loss in animals is reminiscent of the shape of growth curves for microorganisms. That this portion of the curve may indeed be nothing but a growth curve is suggested by the data in TABLE II which reveal that diseased animals stop all movement, and begin to blacken before the steepest portion of the curve is reached, so that weight changes after this point may be determined by the metabolism of the fungus alone.

In the light of these considerations, it is probable that the most reliable index of the effect of temperature upon the host-parasite relation may lie in that portion of the temperature-weight loss curve which immediately precedes the steep rise in the rate at which weight is lost. It is in this part of the curve that we can be most certain that we are measuring an effect involving both the host and parasite, rather than one involving either one alone.

It is suggested, then, that quantitative studies of virulence can be based on respiratory gas exchange and on weight loss in the insect pupa during disease. These techniques, plus the ones discussed in a previous paper (Sussman **1951a**) wherein it was found possible to calculate the MLD for a fungus infection, make it possible to pursue genetic and physiological experiments on quantitative aspects of the disease relationship.

SUMMARY

1. Diseased pupae of *P. cecropia* undergo a 20-fold increase in oxygen uptake as a result of parasitism by *A. flavus;* a substantial part of this increase is due to the metabolism of the host alone.

2. Weight loss of diseased pupae roughly parallels the respiratory increase and is due mostly to evaporation of water from the spiracles.

3. The rate of weight loss is directly proportional to the temperature up to 28° C.

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SOME WATER RELATIONS OF ERYSIPHE POLYGONI CONIDIA

C. E. YARWOOD [1]

(WITH 6 FIGURES)

Powdery mildews (Erysiphaceae) are unique among plant pathogens in that the conidia of many or all of them will germinate under dry conditions which would prevent the germination of most fungus spores (**3**, **4**, **6**, **7**, **8**, **13**). *Erysiphe polygoni* conforms to the general characters of the group and is one of the most important, and most extensively and easily studied. Brodie and Neufeld (**4**) concluded that the conidia of *E. polygoni* contained little water and that they did not shrink during germination. Both of these conclusions disagree with those of the present writer (**13**, **15**). I acknowledge the incompleteness of my earlier data and report herein further studies of the xerophytism of this species.

STRUCTURE OF CONIDIA

Powdery mildew conidia are among the largest one-celled spores of the fungi. Other characters by which they appear to differ from most other air-borne spores are their thin, smooth, and hyaline walls, their turgid condition when fresh (even when examined in the dry condition), their large vacuoles, and the active Brownian movement of their protoplasm. They are fragile, and can be easily broken open and smeared on a glass slide with a fine transfer needle. Their internal contents (see FIG. 1) can be seen well when the spores are immersed in water and examined with the oil immersion objective of the phase contrast microscope. Most of the spore consists of vacuoles, which may be as few as 3 or as many as 25. The vacuoles have been observed to fuse within the spore, but the

[1] The aid of Morris Cohen in making most of the measurements of density and in suggesting the equation to determine the water loss from germinated and non-germinated spores is gratefully acknowledged.

reverse, a subdivision of vacuoles, has not been seen. While many vacuoles are spherical they are sometimes distorted by apparent lateral compression. In the vacuolar sap of fresh spores fine particles in an active state of Brownian motion can be seen but their shape cannot be resolved. After the spores have been in water an hour or so much larger particles can be seen in the vacuoles. Bacterium-like particles have been seen in the vacuoles on several occasions. Between the vacuoles, and lining the internal periphery of the spores, is relatively dense protoplasm which shows less active Brownian movement than the particles within the vacuoles. The large single nucleus with nucleolus was usually centrally located and could usually not be distinguished in the unstained spore. Spores frequently burst after being in water for an hour. The

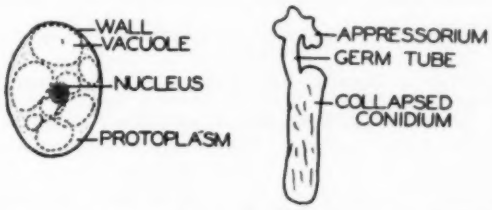

FIG. 1. Conidia of *Erysiphe polygoni* from bean. Left: mature conidium. Right: germinated conidium showing collapsed condition of spore after germination in dry air.

protoplasm and vacuoles sometimes emerged through a terminal pore and sometimes through a lateral rupture in the wall. The peripheral protoplasm scattered in the water, but the Brownian movement of the mostly spherical particles usually became more intense than it was within the spore. Many of the vacuoles also retained their identity after the spores burst and fusion of vacuoles after bursting of spores has been seen. This might indicate that the vacuoles were of a non-watery nature, or that they possessed some boundary layer which was not miscible with water.

Moisture content of conidia by heat of solution of sulfuric acid. —Since previous estimates of moisture content of spores by the conventional dry weight method (**15**) yielded conclusions contradictory to those Brodie and Neufeld (**4**) secured by a staining

method, it would be desirable to estimate the water content of *E. polygoni* spores by a third independent method. In unpublished results the writer has found a useful correlation between the heat of solution of sulfuric acid with various natural materials and the water content of the same materials by the conventional dry weight method. This method has been applied to *E. polygoni* conidia and results are presented in TABLE I. For samples which gave an average of 72 percent water by the dry weight method, the water content by the heat of solution with sulfuric acid was 66 percent. This method also indicates the spores have a high water content.

DENSITY OF CONIDIA

Density is a useful absolute character of any body and may be an index of its water or oil content. Brodie and Neufeld's con-

TABLE I

WATER CONTENT OF *Erysiphe polygoni* CONIDIA AS DETERMINED BY THE CONVENTIONAL DRY WEIGHT METHOD, AND FROM THE HEAT OF SOLUTION OF SULFURIC ACID [1]

Sample[2]	Fresh weight of spores, grams	Dry weight of spores, grams	Ml. of H_2SO_4 used	Maximum increase in temperature of spores plus acid over original temperature of acid °C.	Ml. of water corresponding to increase in temperature[3]	Percent water
1a	0.4688	0.1314				72
b	0.5478		10	13.1	0.381	70
2a	0.2328	0.0662				72
b	0.2720		5	13.4	0.205	74
3a	0.6653	0.1772				73
b	2.340		100	6.7	1.25	53
4b	0.210		10	5.2	0.14	67

[1] No correction is made for the heat of solution of dried spores with sulfuric acid, or the heat loss as the mixture of acid and spores slowly reached its maximum temperature. The heat of solution of dried spores in 10 cc. sulfuric acid was about 0.58° C. for each 0.1 g. dry weight of spores. For sample 1b the heat of solution for the dry matter in the spores would be about 0.9° C. or the corrected temperature increase would be 0.9° C. lower than observed, or about 12.2° C. This would not greatly change the value for percent water in the spores. The rate of heat loss was not determined but correction for this error would raise the value for water content of spores as determined with sulfuric acid.

[2] Sample numbers were taken on different days, but a and b for each number were portions of the same composite sample.

[3] Determined by interpolation on a graph of the relation of the amount of water added to sulfuric acid and the temperature of the mixture.

clusion (**4**) that conidia of *Erysiphe polygoni* have a low water content and highly concentrated protoplasm would indicate they might have a high density, while my conclusion (**15**) that *E. polygoni* conidia have a high water content would indicate the reverse. The determination of density is essential to the calculation of surface area of spores to be used later in this paper. Previous determinations of the density of powdery mildew conidia have not been found, but records found for spores of certain other fungi are given in Table II.

Three methods of measuring the density of spores were tested. In the first the spores were suspended in sugar or salt solutions of known density and their rise or fall noted in a horizontal microscope. Results of such tests with conidia of *Erysiphe polygoni*, summarized in FIG. 2, indicate a range in density of 1.09 to 1.12. For spores tested in sucrose solutions the density was apparently

TABLE II

DENSITY OF VARIOUS AIR-DISSEMINATED SPORES

Species	Type of spore	Method of determination[1]	Density of spores	
			Range of values	Average
Coprinus plicatilis	basidiospores	rise or fall		1.21[2]
Psalliota campestris	basidiospores	rise or fall		1.2[2]
Amanitopsis vaginata	basidiospores	rise or fall		1.02[2, 3]
Polytrichum sp.	[4]	volume:weight		1.53
Lycoperdon sp.	[4]	volume:weight		1.44
Lycopodium sp.	[4]	volume:weight		1.175
Erysiphe polygoni	conidia	rise or fall		1.09[5]
Erysiphe polygoni	conidia	rise or fall	1.09–1.12	
Erysiphe polygoni	conidia	rate of fall	1.01–1.14	1.094[6]
Erysiphe polygoni	conidia	volume:weight	1.04–1.27	
Uromyces phaseoli	uredospores	rise or fall	1.42[7]	
Uromyces phaseoli	uredospores	rise or fall	1.52[8]	
Uromyces phaseoli	uredospores	rate of fall	1.09–1.29	1.36
Uromyces phaseoli	uredospores	volume:weight	1.34–1.35	
Peronospora destructor	sporangia	rise or fall	1.17[7]	
Peronospora destructor	sporangia	rise or fall	1.48–1.54[8]	1.34

[1] Details of methods given in text.
[2] Data from Buller (**5**) given as specific gravity.
[3] Spores contained much oil.
[4] Data for moss spores given by Zeleny and McKeehan (**17**).
[5] Data of *E. polygoni* from clover, see Fig. 3.
[6] Data of *E. polygoni* from bean, see Fig. 3.
[7] Immersed in sucrose solution.
[8] Immersed in potassium carbonate solution.

lower for spores produced on detached leaves in a petri dish than for spores produced on greenhouse plants. This may be because the high humidity in the dishes favored a higher water content of the spores. For spores produced on greenhouse plants the density was apparently lower when tested in K_2CO_3 solutions than when tested in sucrose solution. Microscopic examination showed that K_2CO_3 caused some distortion of the spores whereas sucrose did not.

Another method of determination of density of spores was to measure their rate of fall in water and to calculate their density

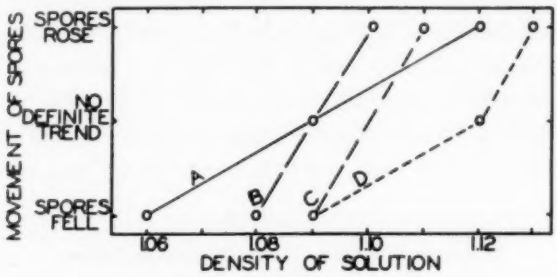

FIG. 2. Density of *Erysiphe polygoni* conidia as determined by their rise or fall in solutions of known density. A. Spores from detached bean leaves on 10 percent sucrose solution in petri dishes. Rise or fall determined in sucrose solution. B. Spores from detached clover leaves on 10 percent sucrose solution in petri dishes. Rise or fall determined in sucrose solutions. C. Spores from greenhouse bean plants. Rise or fall determined in solutions of potassium carbonate. D. Spores from greenhouse bean plants. Rise or fall determined in sucrose solutions.

from Stokes' law (**11**). The time for 26 conidia of *Erysiphe polygoni* from bean to fall 1500 μ in water at 25° C. ranged from 15 to 106 seconds and averaged 52 seconds. The average velocity was 2.9×10^{-3} cm./sec. The average size of 10 of these spores measured in water was 34.5×20.1 μ or their average geometric mean radius was

$$\frac{\sqrt{34.5 \times 20.1\mu}}{2} = 13.1\mu.$$

From Stokes' law,

$$\text{density of spores} = \frac{9\ Vu}{2\ gr^2} + \sigma,$$

when V = terminal velocity,
u = viscosity of medium,
g = acceleration due to gravity,
r = radius of body,
σ = density of medium.

By substitution,

$$\text{density of spores} = \frac{9\times2.9\times10^{-3}\times8.94\times10^{-3}}{2\times980\times1.31\times1.31\times10^{-6}} + 0.997 = 1.067.$$

The third method was to add fresh spores to a weighed and calibrated vial containing xylol, and to measure the increase in weight and in volume. Weight was measured on an analytical balance and volume was determined by measuring with a horizontal microscope the distance between the xylol meniscus before and after adding the spores. Density of spores was then calculated as

$$\frac{\text{change in measured weight of vial}}{\text{change in measured volume of liquid}}.$$

In 6 determinations with 0.09 to 0.29 grams of spores per determination, density values of 1.040 to 1.275 and averaging 1.113 were secured.

In order to compare powdery mildew conidia with air borne spores which are believed to require external free water for germination, the density of sporangia of *Peronospora destructor* and of uredospores of *Uromyces phaseoli* were measured by the above methods. Results with these fungi and density measurements of other spores are summarized in Table II. With *Uromyces* the rate of fall method gave significantly lower values than the rise or fall method. This may be because the numerous spines on the spores, which are included in the measurements of dimensions, greatly slowed down the rate of fall of these spores in comparison with spores of the same size without spines.

Of the three methods of measuring density, probably none was adequately tested, but the writer believes that where lots of 0.1 gram or more of spores are available, the volume : weight method is probably the easiest. When the amount of spores is small the rise or fall method is perhaps the best, and sugar solutions appear preferable to salt solution.

Of the 9 organisms for which spore density data are available, *E. polygoni* has the lowest density except for *Amanitopsis*, which, according to Buller (**5**), contains much oil. The low density of *E. polygoni* conidia would indicate, though not conclusively, that they have a high water content, probably significantly higher than that of most other fungi for which data are available.

WATER LOSS FROM CONIDIA

If spores need water for germination and yet *Erysiphe polygoni* conidia germinate in a dry environment, they must contain considerable water and lose it slowly. Other spores which have been tested apparently lose their water quickly in air (**5**) and if Buller's information is correct, they probably lose it too quickly to permit the rate to be measured satisfactorily by conventional methods. According to Buller's data for *Amanitopsis vaginata* in his Fig. 63, and on the basis of several unverified assumptions, it may be deduced that these spores lost about one-third of their weight and most of their free water in the first 10 seconds of fall in air. With *Erysiphe polygoni* conidia on the other hand, the rate of decrease of weight of the spores, which is presumed to be due to loss of water, can be followed accurately by conventional methods.

Size and weight of conidia.—Conidia of *E. polygoni* from bean are prolate spheroids with a length (l) of 35 μ and a diameter (d) of 20 μ. The volume of a single spore is therefore $0.52\ ld^2 = 0.52 \times 35\ \mu \times 20\ \mu^2 = 7280\ \mu^3 = 728 \times 10^{-11}$ cm^3. The weight of a single spore is volume $\times$ density $= 728 \times 10^{-11} \times 1.09$ grams $= 792 \times 10^{-11}$ grams. The surface of a single spore is $3.14\ l^2S = 3.14 \times 35\ \mu^2 \times 0.497 = 1910\ \mu^2 = 191 \times 10^{-7}$ cm.^{2} when $3.14\ l^2$ is the surface area of a sphere with a diameter equal to the length of the spore, and S is the surface coefficient (**12**) for determining the surface of a prolate spheroid from the surface of a sphere having a diameter equal to the major axis of the spheroid. The surface area per gram of spores is

$$\frac{\text{area of one spore}}{\text{weight of one spore}} = \frac{191 \times 10^{-7}\ \text{cm}^2}{792 \times 10^{-12}} = 2416\ \text{cm}^2.$$

Since the same number of spores per dish could not be secured in different dishes or in different trials, and since concentration of spores would be expected to affect rate of water loss per spore or per unit area of spores, the relation of size of spore sample to rate of water loss was determined. Spore samples ranging from 0.005 to 0.460 gram measured weight per dish were weighed at intervals and weight loss expressed as water loss per hour per dcm.^{2} surface area of spores. Data for 2 trials, presented in FIG. 3, show that as the concentration of spores increased the rate of water loss per unit surface area of spores decreased, but not proportionately. For

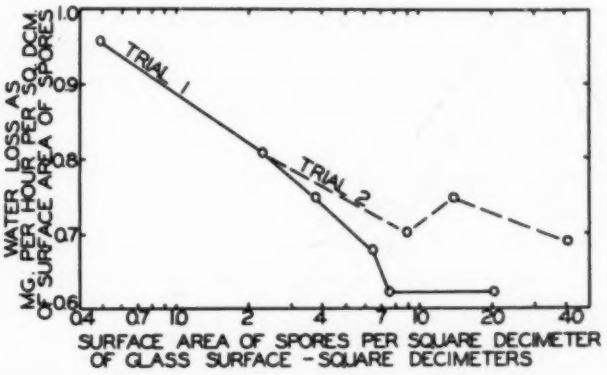

FIG. 3. Relation of size of spore sample to rate of water loss from conidia of *Erysiphe polygoni*. Each sample was uniformly distributed over the bottom of an open petri dish with an inside diameter of 57 mm. As the size of the spore sample increased, the rate of water loss per unit area of spores decreased.

example, a 100 fold or 10,000 percent increase in the number of spores caused a decrease of only 32 percent in the rate of water loss per spore. Therefore with the differences in water loss with which we are concerned in this paper, differences due to size of spore sample was not an important source of error.

Relation of germination to rate of water loss.—When *E. polygoni* conidia in dishes were exposed to daylight in the laboratory their rate of weight loss (= water loss) increased up to 3 to 12 hours and then decreased to zero at about 65 hours (FIG. 4). This increased rate of water loss during the first few hours, not shown by

dishes of water in parallel series, could be because germinating conidia lost more water than non-germinating conidia. In one test two lots of spores were placed in jars under green cellophane (green light favored germination) and two lots were placed in darkness, all being at 22° C. and 44 percent relative humidity. The test continued for 7 hours (9 A.M. to 4 P.M., March 23). In this test the rate of water loss from the water controls in the same environment was 170 milligrams per hour per square diameter (mg./hr./dcm.^{2}) in the light and 173 mg./hr./dcm.^{2} in the dark. These rates are

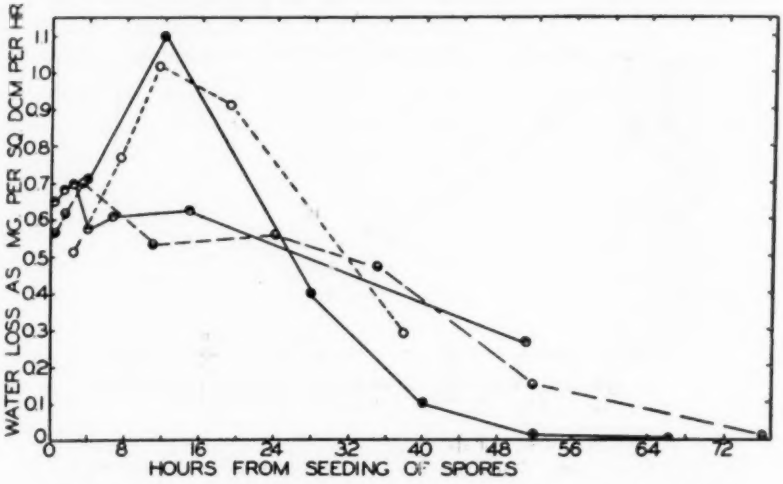

FIG. 4. Water loss from *Erysiphe polygoni* conidia as measured by weight loss in 4 trials. The average rate of water loss was about 0.6 mg./hr./dcm.^{2} during the first hour, 0.8 mg./hr./dcm.^{2} for the 12th hour, and 0 for the 75th hour.

not likely significantly different from each other, but are a great deal less than for water in the open laboratory. In the green light 89 percent of the spores germinated and in the dark 19 percent. Water loss from the spores was 0.71 mg./hr./dcm.^{2} in the green light, and 0.47 mg./hr./dcm.^{2} in the dark. On the assumption that water loss from germinated spores was the same per spore in light and in darkness and that water loss from non-germinated spores was the same in light and in darkness, then the above values may be corrected to 0 and 100 percent germination as follows:

Let x be water loss by germinated spores and y be water loss by non-germinated spores. Then:

$$89x + 11y = 0.71 \text{ mg./hr./dcm.}^2$$
$$19x + 81y = 0.47 \text{ mg./hr./dcm.}^2$$

Solving the two simultaneous equations we get

$$x = 0.00749$$
$$y = 0.00405$$

or germinated spores lost 85 percent more water than non-germinated spores. This value is in reasonable agreement with the value secured by volume measurement to be presented later.

Absolute and relative rates of water loss from spores.—In 5 trials the weight loss from a free water surface and from spores was simultaneously followed in several replications and over several weighing periods. Since the weight loss of spores declines continuously after an initial rise (FIG. 4) the average value for the first 5 hours is fairly typical of that for the first 24 hours. In the prevailing laboratory environment of about 22° C. and 50 percent relative humidity, the absolute rate of weight loss from a water surface ranged from 705 to 890 mg./hr./dcm.^{2} in 4 trials where the dishes were in the open laboratory, and averaged 170 mg./hr./dcm.^{2} for dishes in the sealed glass jars previously described. In the same trials the absolute water loss from conidia during the first 5 hours after detachment ranged from 0.537 to 0.744 mg./hr./dcm.^{2} and the value of 0.713 for spores in sealed jars was not significantly different from those in the open laboratory. Excluding the data for dishes placed in jars, the water loss from spores in these trials ranged from 0.00068 to 0.00092 times the rate of water loss from a free water surface, or spores lost water at less than one thousandth the rate of water loss of a free water surface. The slow rate of water loss from spores is even impressive if compared with the rate from leaves. Water loss from untreated leaves of various greenhouse plants was about one-fourth that of a free water surface (**16**), and alfalfa growing outdoors (**2**) lost water at about one-third that of a free water surface. If we accept one-third as a useful value then the rate of water loss from *Erysiphe polygoni* conidia would be about one four-hundredth of that from a leaf

surface. This lends strong support to the idea that increased water loss from plants as a result of mildew infection is principally due to water loss from the host and not from the fungus (**14**).

SHRINKAGE OF CONIDIA AS OBSERVED MICROSCOPICALLY

Conidia shaken from greenhouse plants to dry slides were measured in air at 400 diameters with an eyepiece micrometer. In

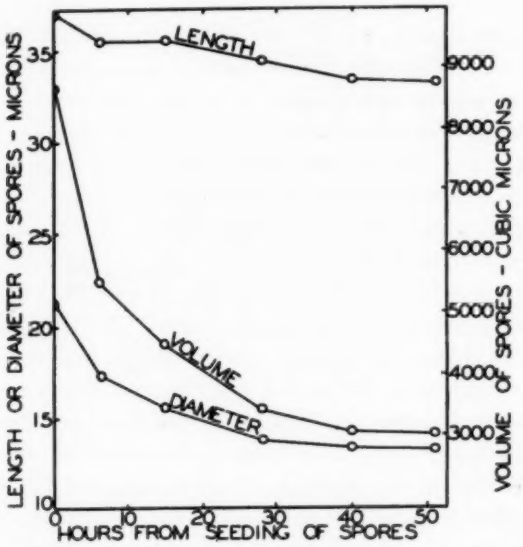

FIG. 5. Changes in length, diameter, and volume of 12 conidia of *Erysiphe polygoni* during drying and germination March 28, 1950. During the 50 hours of this test the length of the conidia decreased by about 10 percent, the diameter decreased by about 39 percent, the diameter squared decreased by about 63 percent, and the volume decreased by about 65 percent. Reduction in volume was therefore principally and roughly proportional to the reduction in the square of the diameter.

earlier trials a standard eyepiece micrometer with 1 division = 3.6 μ was used and measurements were estimated to 0.1 eyepiece division. All later trials were made with a filar micrometer. Spore germination was allowed to proceed and spore size was measured in the same environment, which was usually about 22° C. and 50 percent relative humidity.

As germinating or non-germinating conidia of *E. polygoni* dried out, they changed from prolate spheroids to a more cylindrical shape with rounded ends (FIG. 1). Length was only slightly reduced but diameter was greatly reduced (FIG. 5). Since volume decreased as the square of the diameter but directly as the length, the changes in measured length had only a slight effect on spore volume. With the spores averaged in FIG. 5, the original volume decreased from 8650 μ^3 to 3035 μ^3 or by 65 percent of their original volume. Of this reduction in volume, the reduction in length contributed only 9 percent while the reduction in diameter contributed about 56 percent. In most subsequent trials only the diameter of the spores was measured and changes in volume were based on the changes in diameter. This limitation in method introduces no errors of great significance in this study.

Relation of germination to conidium shrinkage.—While germinating and non-germinating conidia of *Erysiphe polygoni* shrink in a dry atmosphere, the germinating spores shrink at a faster rate. Brodie and Neufeld (**4**) have criticized my previous data (**13**) on spore shrinkage due to germination on the grounds that my measurements were made at 5, 10, and 24 hours after seeding the spores, and Brodie and Neufeld believe that growth ceases at about 4 hours and that the conidia are dead before shrinkage occurs. I agree with Brodie that with powdery mildew conidia, unlike most other fungus spores, either germination occurs soon after detachment or the spore dies without germinating. Also there are important differences between different strains of the same species. The conidia of *E. polygoni* from clover germinate more promptly than those from bean. In addition to the writer, Hammerlund (**9**), Pratt (**10**) and Berwith (**1**) and probably others have recorded initiation or continuation of germ-tube growth of powdery mildews at more than 4 hours after detachment. While these investigators did not make their records from spores on dry slides it is not felt that this difference should affect the validity of their observations. Logically also it is difficult to understand how a large, thin-walled cell containing an abundance of water, or some volatile fluid with some of the characteristics of water, can fail to shrink when it displaces part of its substance into a germ tube, and loses part of its weight continuously.

In spite of *a priori* objections to Brodie and Neufeld's ideas on spore shrinkage, their frequently cited conclusions, the incompleteness of the writer's previous data, and the absence of other information confirming either Brodie or Yarwood, make desirable a revaluation of the subject. In the original report (**13**) only data for germinated spores were included. The present study, with a different morphologic and biologic strain of the fungus, in a different

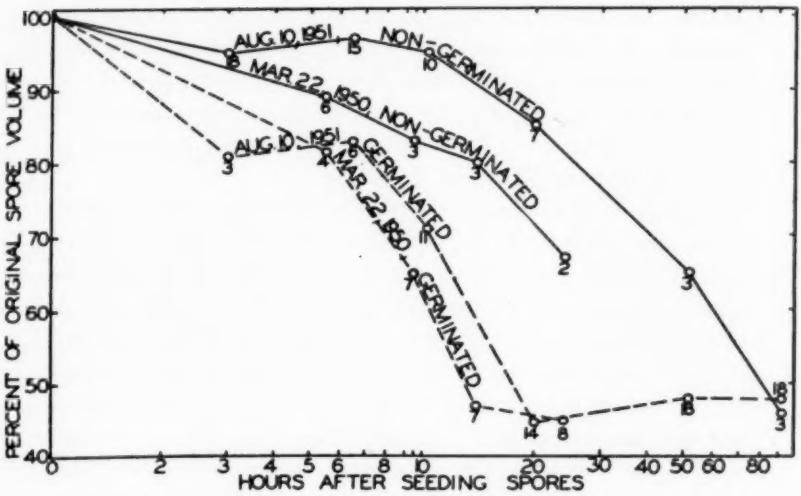

FIG. 6. Changes in volume of germinated and non-germinated conidia of *Erysiphe polygoni* in a dry atmosphere. There were 21 conidia in the August 10 observations and 10 in the March 22 observations. The numbers along and below the graphs refer to numbers of spores in the stated condition at the time of record. On the average, germinated spores had decreased in volume by 32 percent in 10 hours whereas non-germinated spores had decreased in volume by only 12 percent during the same period.

locality, and with simultaneous data on germinated and non-germinated spores, should be more conclusive. Results of two typical trials are given in FIG. 6.

In the trial of March 22, 1950, fresh, turgid, non-germinated conidia were shaken from greenhouse plants on to dry slides in a well-lighted laboratory at 22° C. and 45 percent relative humidity. At 7 A.M. the diameter of 10 positioned spores was measured, and at intervals thereafter the same spores were again measured and

their germination noted. Only 4 spores had germinated after 5 hours, and 4 more germinated in the next 24 hours. Results for 14 hours are perhaps most impressive. At this time germination was still not complete and it is reasonable to assume the spores were still alive, yet the 7 germinated spores had shrunk by 54 percent of their original volume, while the 3 non-germinated spores had shrunk by only 21 percent. In the trial of August 10, 1951, with germination incomplete at 10 hours, the germinated spores had shrunk by 29 percent while the non-germinated spores had shrunk by only 5 percent. The apparent rise in spore volume of germinated and non-germinated spores from 3 to 6 hours in the August 10 trial remains unexplained but has been observed several times for individual spores. This might be considered as evidence in support of Brodie's idea that "The high osmotic pressure might be an aid to the spores in absorbing water from relatively dry atmospheres. . . ." In the trial of August 10, new germinations occurred between 20 and 53 hours after seeding, which is at least 5 times the maximum germination time indicated by Brodie and Neufeld.

While the quantitative data on the measured change in volume of individual spores are felt desirable in this study, they are not regarded as essential in reaching the conclusion that germinated spores shrank more than non-germinated spores. This difference is of such a magnitude that it can be seen without measurements, and has been confirmed by microscopic observation of the writer's material by three independent and presumably unbiased observers.

Relation of humidity to shrinkage of conidia.—If the loss in weight and volume of conidia during germination in a dry environment is due to loss of water, then such loss should be less in a humid environment, while if shrinkage were due to the loss of some non-aqueous volatile substance, it should proceed independently of humidity. In many trials no clear loss in weight or microscopic shrinkage of germinated or non-germinated spores has been observed in a humid atmosphere in up to 60 hours, whether the spores were on the surface of water or on the surface of glass in moist chambers. This is considered to support the thesis that the shrinkage of germinating or non-germinating spores in a dry environment is due to loss of water. The bursting and partial

collapse of some spores immersed in water cannot readily be confused with the shrinkage under discussion here.

Relation of germ tube length to shrinkage of conidia.—It would be expected that in the absence of absorption of water or nutrients by the spores during germination, the formation of a germ-tube would cause a shrinkage of the spore due to translocation of its contents, and that the shrinkage of the spore would be proportional to the size of the germ-tube. Also it might be expected that if water loss from the spore and germ-tube was eliminated as a cause of spore shrinkage, the reduction in volume of the spore due to formation of a germ-tube would be more closely correlated with germ-tube length than if the spores were in a dry environment where loss of water would cause considerable shrinkage in the absence of germ-tube formation. In measurements of spore diameter and germ-tube length for 22 spores in a moist chamber and for 36 spores in a dry chamber, only a poor correlation was established between the size of the germ-tubes and the volume of the spores. The difficulty of establishing such a relation may be in part because the germ-tubes formed from powdery mildew conidia on glass slides constitute only a small fraction of the total volume of the spores. Brodie (**3**) has estimated the volume of the germ-tubes of *Erysiphe polygoni* as only 3.3 percent of the volume of the mother spore. A change of volume of this magnitude could not be accurately measured by the methods used in this study.

SUMMARY

The distinctive morphological characters of the conidia of *Erysiphe polygoni* are their large size, fragility, large vacuoles, thin walls, and the active Brownian movement of particles in the protoplasm and vacuoles. The weight, volume, and surface area per spore were determined as 792×10^{-11} grams, 728×10^{-11} cm.^{3}, and 191×10^{-7} cm.^{2} respectively.

The density of conidia of *E. polygoni* was measured by their rise or fall in solutions of known density, by their rate of fall in water, and by the relation between volume and weight of a measured mass of spores. The average value was 1.094. The density

of *Uromyces phaseoli* uredospores and *Peronospora destructor* sporangia was determined as 1.36 and 1.34 respectively.

The moisture content of *E. polygoni* conidia was determined as 72 percent by the conventional oven dry weight method and 53 to 74 percent by the heat of solution of the spores in sulfuric acid. A high water content might also be indicated by their low specific gravity and their lack of shrinkage in a humid atmosphere.

In a laboratory environment where a free water surface lost water at about 800 mg./hr./dcm.^{2}, conidia of *E. polygoni* lost water initially at about 0.640 mg./hr./dcm.^{2}, or spores lost water at about 0.08 percent of the rate from a free water surface. This indicates an efficient mechanism of water retention of the spores. Germination continued even after the mass of the spores had lost 72 percent of its water content at 20 hours after seeding. Water loss from spores rose to a maximum rate at about 8 hours after seeding and fell to zero at about 72 hours after seeding. A 10,000 percent increase in the amount of spores per unit area caused only a 32 percent decrease in the rate of water loss per spore.

The relative rate of water loss from germinated and non-germinated spores was determined by comparing the weight loss of a spore sample in green light showing a high percentage of germination with the weight loss of a spore sample in darkness showing a low percentage of germination. Germinating spores lost 85 percent more weight than non-germinating spores in 7 hours.

The relative rate of shrinkage of germinating and non-germinating spores was determined by measuring microscopically and at intervals the diameter of individual spores some of which germinated and some of which did not. Germinating spores decreased in volume at about 3 times the rate of non-germinating spores.

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A PHYSIOLOGICAL STUDY OF THE FUSARIA CAUSING TOMATO, CABBAGE AND MUSKMELON WILTS [1]

HELEN MOORE AND CHARLES CHUPP

(WITH 1 FIGURE)

The vascular wilt diseases caused by species of *Fusarium* in the section *Elegans* are so widespread and of such economic significance that it is not necessary to emphasize the importance of properly placing these fungi in their correct taxonomic positions. As Snyder and Hansen (**14**) point out, the principal difficulty encountered in the classification of the genus *Fusarium* is the wide variations in morphological and physiological characters exhibited by its members. Since most of the known pathogens of section *Elegens* are morphologically alike they "reduced all former species in section *Elegans* to synonymy with *F. oxysporum* (Sch.) Snyder and Hansen, with a 'forma' (Snyder and Hansen) name attached to that of the species to indicate relative specificity for host as well as pathogenicity."

The purpose of the present investigation was a further study for distinguishing species of fungi by methods that might reveal more conclusive evidence either for placing all the strains of section *Elegans* into one species, as advocated by Snyder and Hansen, or for clearly demonstrating the formae in section *Elegans* as sufficiently different to be given species rank.

CULTURAL STUDIES [2]

Nutritional requirements

The nutritional requirements of *Fusarium* species are not at all restricted. Apparently any one of a multitude of carbon and

[1] A revision of a thesis submitted by the senior author to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Master of Science.

[2] Acknowledgment is hereby made and thanks extended to all those who kindly furnished *Fusarium* cultures for these experiments. R. W. Samson,

nitrogen sources may be used without affecting growth to any great degree. The methods used here follow closely those in the Manual of Methods for Pure Culture Study of Bacteria (**1**) since it was thought that techniques employed in the identification of bacteria might also be used in the identification of fungi.

The following tests mostly recommended by the Manual mentional above all resulted negatively in that no distinct differences were shown by the formae used in this study:

1. Although 14 carbon compounds were tested (**1** and **2**), none of the formae could be differentiated on the basis of utilization of any of these carbon sources. All of the 16 isolates used grew well.

2. In the starch hydrolysis trials when the fungi were grown on beef-extract agar, containing 0.2 per cent soluble starch, all of them hydrolyzed starch to the same extent.

3. None of the isolates reduced nitrates to nitrites.

4. All the cultures were able equally to utilize urea in their metabolism with the consequent production of ammonia.

5. In the Gnezda oxalic acid test (**6**) indol was not produced by any of the formae.

6. No hydrogen sulfide was liberated from sulphur containing proteins.

7. All the isolates liquefied a 12 per cent plain gelatin medium with approximately the same rapidity.

8. Pectin was reduced uniformly in all the cultures grown on a sodium ammonium pectate medium (**3**).

9. All the formae reacted similarly on sterile litmus milk in the precipitation of casein and peptonization until the milk was clear and light brown in color.

10. In the actidione test as given by Gottlieb, Hassan and Linn (**8**) there were few specific differences in reaction among the various isolates after one week on potato dextrose agar containing the

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Purdue University, A. G. Plakidas, Louisiana State University and Agricultural and Mechanical College, L. J. Alexander, Agricultural Experiment Station, Wooster, Ohio, R. E. Wilkinson and A. G. Newhall, Cornell University, furnished cultures of *F. oxysporum lycopersici*. H. M. Munger furnished cultures of *F. oxysporum nivcum* and *F. oxysporum conglutinans* in addition to the ones isolated by the senior author.

antibiotic substance. In general, growth was reduced markedly at 20 p.p.m. and was almost completely suppressed at 50 p.p.m.

An experiment was set up to determine the thiamin requirements of the *Fusarium* cultures. The following substances were added to 200 ml. distilled water and autoclaved at 10 pounds pressure for twenty minutes to dissolve the agar: $MgSO_4 \cdot 7H_2O$, 0.05 gr.; KH_2PO_4, 0.15 gr.; asparagin (recrystallized), 0.40 gr.; and agar (special purified), 4.00 gr. To the melted agar was added 10 gr. C.P. glucose. Five-tenths of a milliliter of distilled water was added to one-half of the medium and 0.5 ml. vitamin B_1 (25 milligrams of thiamin per liter) was added to the other half. Thoroughly cleaned Beadle-Tatum fungus growth rate tubes were filled and autoclaved for 20 minutes at 10 pounds pressure. After the agar had hardened the tubes were inoculated and incubated at 27° C. for about a month. A record was kept of the growth rate.

A comparison between the amount of growth on vitamin B_1 synthetic agar and on synthetic agar plus water showed that vitamin B_1 is not essential for the growth of these formae. The cabbage isolates did not produce any pigment in the media nor in the mycelium while in the dark but upon subsequent removal of the growth tubes from the incubator to the laboratory, a faint orange color developed. The tomato and melon isolates were rosy-red with a lavender substrate in the incubator and remained the same color when exposed to light.

Pigment production

Reinking and Wollenweber (**13**) advise growing species of *Fusarium* on rice media to group them into their respective sections according to color reactions. Leonian (**11**), while studying variability and dissociation in the genus, found a variation in pigment formation by growing the fungi on various types of media. Leonian's experiments were repeated in the present study and some other types of media were used. The consistent difference exhibited by the isolates seemed to be the lack of pigment by *F. oxysporum f. conglutinans* on malt extract, potassium nitrate, peptone, oat-

meal and potato dextrose agars, asparagin medium, steamed rice, fresh tomato, and fresh cabbage.

The diagnosis of species by means of growth-inhibiting substances

An experiment was set up to indicate possible variation in the sensitiveness of the *Fusarium* isolates to various concentrations of sodium chloride in a nutrient broth solution. Tubes of 5, 7, 10, and 15 per cent salt solutions were inoculated, incubated at 27° C. and inspected after two weeks. Similarly dextrose was added to a basic salt solution (**1**) to make 0.1 M, 0.5 M, 0.8 M, 1 M, 2 M, and 3 M concentrations and the tubes likewise were inoculated.

Growth gradually ceased as the concentration of NaCl and dextrose became higher. *F. oxysporum f. conglutinans* did not seem to grow as well at any concentration as did the other two formae. The pigment production was good for the tomato and melon isolates up to the 1 M sugar concentration, was less at 2 M and completely absent in the 3 M tubes. The cabbage *Fusarium* organism did not produce pigment in any of the tubes.

A duplication of Coons and Strong's (**4**) work using malachite green and crystal violet as inhibiting substances showed some variations from their results. Only three tomato isolates and one muskmelon isolate exhibited growth at the 1 : 66,666 dilution of malachite green. This would immediately take the tomato *Fusarium* out of Coons and Strong's "sensitive to malacite green" group and put it in the "extremely sensitive to malacite green" classification.

Growth of the melon and tomato formae on crystal violet substantiates the Coons and Strong provisional key with the exception of one tomato isolate which exhibited growth in the 1 : 3333 dilution. The cabbage isolates, however, did not grow at all at 1 : 5000 dilution even though the Coons and Strong key suggests that growth extends to a concentration of 1 : 3333.

It is concluded that, although broad groups of *Fusaria* may be separated by the use of crystal violet and malachite green in the culture medium, positive identification of the organisms causing wilts on cabbage, muskmelon and tomato is dependent upon further

investigation into the range of growth on various concentrations of dye.

Effects of plant extracts on fungus growth

An explanation for the variation in susceptibility to *Fusarium* wilt displayed by various varieties of vegetables may be due to the plant's ability to produce a substance which either neutralizes the fungus toxin directly or inhibits fungus growth. Gottlieb (**7**) found that sap expressed from three varieties of tomato, Bonny Best, Marglobe and Pan American, retarded the growth of *Fusarium oxysporum f. lycopersici* in proportion to the wilt resistance of the variety.

Irving, Fontaine and Doolittle (**9**) identified a substance in the tomato plant, tomatin, which they considered inhibited the growth of the *Fusarium* organism on tomato. They (**10**) reported that tomatin, occurring in *Lycopersicon pimpinellifolium* (Red Currant tomato) and used at the rate of 5 units/ml., effectively inhibited the growth in culture of *F. oxysporum f. lycopersici, F. oxysporum f. pisi* and *F. oxysporum f. conglutinans.*

Fisher (**5**) expressed juice from Bonny Best and Marglobe tomato plants, sterilized it and inoculated it with *F. oxysporum f. lycopersici.* He found that if the Marglobe juice were autoclaved, the fungus grew on it normally as it did on the control test medium. However, if the juice were not heated, growth was inhibited. The fungus grew on Bonny Best juice under any treatment.

In this study an experiment was set up to study the inhibition of growth on all the *Fusarium* isolates by plant juices from both resistant and susceptible varieties of tomato, cabbage and melon. Mature, well-washed plants of Pan American, Valiant and Bonny Best tomato, Iroquois and Bender melons, Danish Strain A cabbage and Danish Ballhead cabbage were ground up and the juice strained through a fine cheesecloth. The juice was then centrifuged, the supernatant liquid sterilized by passage through a Seitz filter, and portions inoculated with *Fusarium* isolates. One milliliter portions were mixed also with nutrient broth agar (9 ml.) and petri dishes were poured.

F. oxysporum f. conglutinans did not grow as well in the pure tomato plant extract as did the muskmelon and tomato Fusaria. This also was true when the plant juice was mixed with the nutrient

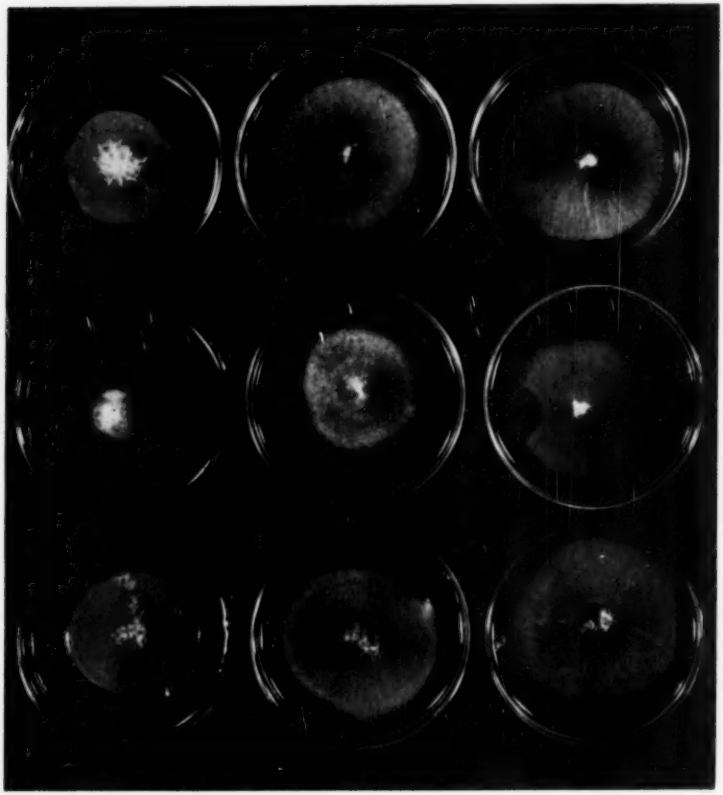

FIG. 1. The inhibition of growth exhibited by Fusaria on nutrient agar plus plant extracts. The vertical rows from left to right contain extracts of Valiant tomato, Iriquois melon, and Danish cabbage. The horizontal rows from top to bottom are inoculated with formae *lycopersici*, *conglutinans*, and *niveum*.

broth agar. In FIG. 1 the plates in the left hand vertical column contain nutrient agar plus extract from Valiant tomato plants. The top plate is inoculated with *F. oxysporum lycopersici*, the middle with *F. oxysporum conglutinans* and the bottom with *F. oxysporum*

niveum. Note the growth inhibition of the cabbage organism on extract from the tomato plant.

The lack of growth of the cabbage isolate on nutrient agar containing tomato plant extract does not indicate a lack of nutrient material since the control plate containing no plant extract grew well. It is indicative, however, of the possible presence in the tomato plant of some factor which is inhibitory in its action towards the cabbage *Fusarium*. It is concluded that this method of differentiating species of *Fusarium* by using plant extracts as inhibitory substances may be of value in the diagnosis of species.

Study of the toxic action of the fungus

An experiment was set up to study the toxic properties of the by-products of the tomato, cabbage and muskmelon wilt fungi. Fifty ml. portions of Coons' medium (**5**) in 125 ml. flasks were sterilized and inoculated with the *Fusarium* isolates. After an eighteen-day incubation period the cultures were filtered through a Buchner funnel and tomato, melon and cabbage plants were placed in the filtrates. Observations were recorded after 48 hours. The following varieties were used: Pan American tomato, Danish Strain A cabbage and Iroquois muskmelon which are resistant to *Fusarium* wilt; and Bonny Best tomato, Danish Ballhead cabbage and Bender muskmelon which are susceptible to *Fusarium* wilt. Flasks of uninoculated Coons' medium, distilled water and tap water were used as controls.

In general both varieties of cabbage were the most resistant to the toxicity of the filtrates. The Danish Ballhead variety showed no wilting in any of the filtrates while the Danish Strain A cabbage plants exhibited a slight degree of wilting in three out of six *Fusarium* filtrates.

The Iroquois and Bender melon plants showed partial to complete wilting in all three types of *Fusarium* filtrates. The control plants in uninoculated media, distilled water and tap water showed no wilting.

The tomato plants wilted only slightly in the *F. oxysporum conglutinans* filtrates but showed more positive wilting in the *F. oxysporum lycopersici* and the *F. oxysporum niveum* culture flasks.

Bonny Best tomato plants in the cabbage *Fusarium* culture exhibited no symptoms at all after 48 hours although in 60 hours black necrotic spots had begun to appear. These dark necrotic portions started at the tips and edges of the leaves and, after a day, extended to the whole leaf. Leaf necrosis on Pan American tomato plants in all the filtrates appeared within 48 hours. Although this preliminary experiment is not extensive enough to form definite conclusions, it is felt that there are a sufficient number of differences in the symptoms displayed to demonstrate the physiological differences inherent in the various *Fusarium* organisms used.

SUMMARY

A physiological study has been made of three *Fusarium* formae in the section *Elegans: F. oxysporum f. lycopersici, F. oxysporum f. conglutinans* and *F. oxysporum f. niveum,* with the purpose of determining differential reactions. It was observed that all the isolates utilized arabinose, xylose, glucose, maltose, dextrin, glycerol, mannitol, dulcitol, salicin, ethyl alcohol, sodium tartrate, hippuric acid, malonic acid and sodium citrate as carbon sources.

All the species of *Fusarium* hydrolyzed starch and utilized the ammonium ion, urea, peptone, proteose-peptone and asparagin as nitrogen sources. None produced indol from tryptophane nor hydrogen sulfide from sulphur-containing proteins. Gelatin was liquefied by all isolates, pectate reduced and precipitation of casein and peptonization occurred in milk cultures. The addition of thiamin to a synthetic medium did not affect growth rate to any marked degree.

The use of organic dyes as growth-inhibiting substances showed some promise as a tool in the diagnosis of species. Actidione and various concentrations of sodium chloride and glucose showed little differential action as growth-inhibiting substances. The plant extract from Valiant tomato plant did, however, inhibit the growth of *F. oxysporum conglutinans* on nutrient agar and in that respect some differential action appeared.

An examination of the host plants in fungal filtrates shows that both Pan American and Bonny Best plants wilted only slightly in the cabbage *Fusarium* filtrate. Bonny Best had not begun to

exhibit symptoms in the *F. oxysporum conglutinans* filtrate after 48 hours although black necrotic spots began to appear in 60 hours. With the exception of a faint orange color developed on the synthetic agar used in the vitamin B_1 experiment the cabbage organism failed to produce pigment on any of the media used in the study. The inhibition of the cabbage *Fusarium* organism on a nutrient medium containing extract from a tomato plant indicates a possible method to be used in the diagnosis of species. The lack of wilting by tomato plants in the cabbage *Fusarium* filtrate also suggests a field for future investigation.

A comparison of the physiological characters exhibited by the *Fusarium* forms of this study indicates the possibility that further work may supply a basis for differentiating at least the cabbage strain of *Fusarium* from some of the other forms in the *Elegans* group.

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ANOTHER NEMATODE-STRANGULATING DACTYLELLA AND SOME RELATED HYPHOMYCETES

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

Among the clampless hyphomycetes subsisting by capture of motile eelworms the species that hold and subdue their prey through constriction of hyphal rings invite special interest since they possess mechanically active organs analogous to the trapping devices of such remarkable carnivorous phanerogamic plants as *Dionaea muscipula* Ellis and *Aldrovanda vesiculosa* L. In earlier papers I described nine nematode-strangulating species, assigning them to four form genera in accordance with the commonplace diagnostic features of their conidial apparatus: *Trichothecium polybrochum* (**2**: 535–538) being distinguished by solitary uniseptate conidia; *Arthrobotrys dactyloides* (**2**: 482–487) by clustered uniseptate conidia; *Dactylella doedycoides* (**4**: 448–456), *Dactylella heterospora* (**6**: 339–349), and *Dactylella stenobrocha* (**10**: 2–12) by solitary biseptate conidia; *Dactylella bembicodes* (**2**: 487–492) by mostly solitary triseptate conidia; *Dactylaria brochopaga* (**2**: 514–518) by clustered triseptate conidia; and *Dactylella coelobrocha* (**9**), as well as *Dactylella aphrobrocha* (**10**: 12–22), by solitary quadriseptate conidia. The nematode-strangulating *Dactylaria gracilis*, which Duddington (**11**) recently described in England, produces conidial apparatus much resembling that of *D. brochopaga*—the resemblance being especially close if the spore dimension shown in the figure of the British form are considered. A strangulating species clearly different from any hitherto made known came to light in several maizemeal-agar plate cultures which after being overgrown by mycelium of *Pythium debaryanum* Hesse had been further planted with pinches of leaf mold taken up in deciduous woods

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near Cumberland, Maryland, on February 6, 1951.[2] It was first noticed 17 days after the forest detritus had been added, an ample food supply having meanwhile become available through multiplication of several species of nematodes introduced with the partly decayed material.

When growing in nematode-infested agar plate cultures the new fungus extends its mycelium rather sparsely. The hyphae pursue relatively straightforward courses, often elongating on or close under the surface. They give rise at intervals to predacious rings which like the similar organs of all other actively strangulating forms consist of three arcuate cells and are borne on stalks usually composed of two segments. In its original position the individual ring is commonly oriented in a plane perpendicular to the parent hypha as well as to the surface of the ambient (FIG. 1, *A; B, a;* FIG. 2, *A, a*), and consequently is directed edgewise to the observer when it is being examined under a microscope in slabs taken from an agar plate culture. Some rings, when bustled by large nematodes, are pushed sideways into oblique (FIG. 1, *C–E;* FIG. 2, *B, a*) or horizontal positions (FIG. 1, *B, b; F–H; I, a, b; J–R;* FIG. 2, *C, a, b; D–J*) in which their cellular make-up and their attachment to the stalk are better revealed. Their formative stages (FIG. 1, *T, a, b;* FIG. 2, *K, L*) show a sequence of development generally similar to that illustrated in *Dactylella aphrobrocha* (**10**: Fig. 5, I, a–e). Fully developed rings of the new fungus most closely resemble the homologous organs of *D. doedycoides* and *D. heterospora,* for as in these species the component arcuate cells have a noticeable median protrusion on their concave inner side that gives the aperture an outline suggestive of trefoil ornamentation. As in *D. doedycoides* and *D. heterospora,* again, the supporting stalks are comparatively long and somewhat slender, and the two cells composing them seem usually to differ little in size. This rangy type of stalk differs markedly from the short stout type exemplified in *D. bembicodes,* where the proximal cell most often is conspicuously shorter than the distal cell, its length, indeed, being frequently equal to only half of its own width.

[2] I am greatly indebted to Professor Walter F. Jeffers of the University of Maryland for his kindness in obtaining this material.

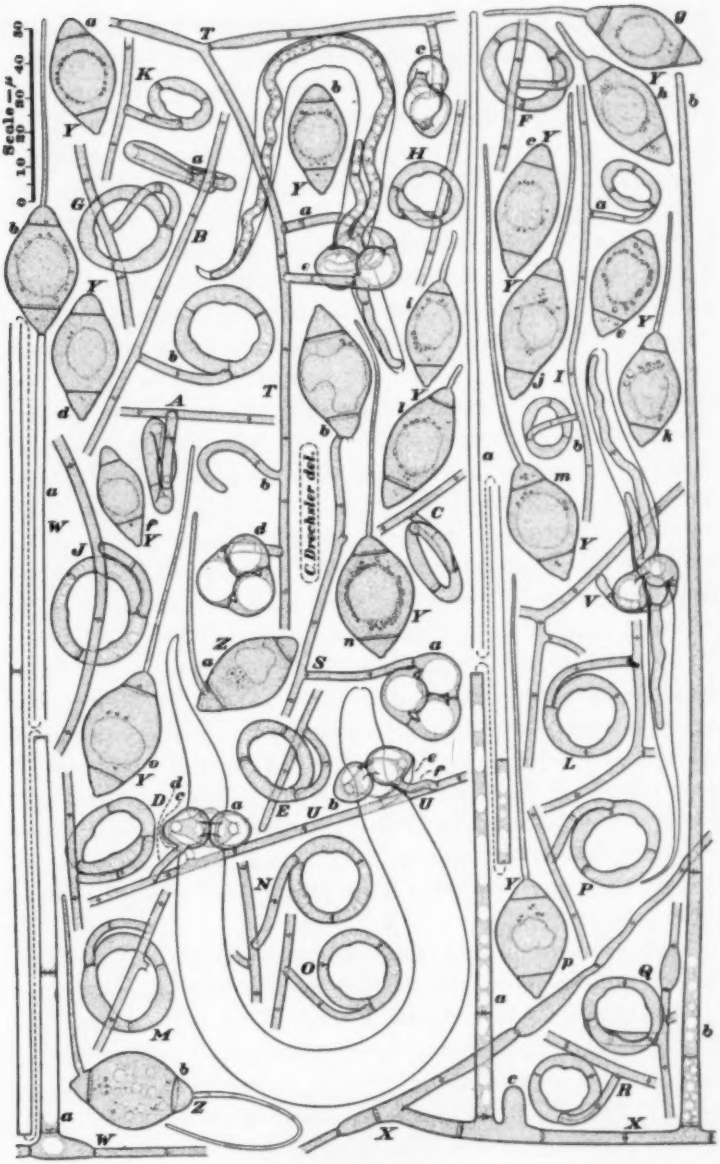

FIG. 1.

Although as in other strangulating species the rings of the new hyphomycete are found here and there to have closed emptily (FIG. 1, *S, a; T, c, d;* FIG. 2, *M, a, b*), they ordinarily operate by closing on the body of an intruding eelworm (FIG. 1, *U, a, b;* FIG. 2, *A, b, c; C, c, d*). After the struggling animal has been brought into submission through the pronounced constriction effected by the ring, its integument is penetrated and assimilative hyphae are extended forward and backward throughout its length (FIG. 1, *T, e; V;* FIG. 2, *B, b*). At first the invading hyphae are obscured badly owing to the globuliferous degeneration of musculature and organs that marks their advance. They become more clearly visible when the fleshy materials have been largely appropriated by them. As the nutrients are depleted more and more, the assimilative hyphae show increasing vacuolization. Eventually when the digestible substance of the captive has vanished entirely, leaving only the empty integument and, in males, the spicula, the assimilative hyphae likewise are evacuated of all living contents through gradual withdrawal of protoplasm backward into the parent hypha. The backward withdrawal of protoplasm takes place by way of the stalk originally supporting the ring (FIG. 1, *T, e; V;* FIG. 2, *A, b, c; B, b*) wherever this longish two-celled connection has not been seriously damaged by the struggles of the animal. In the frequent instances where the original stalk incurred severe injury it degenerates internally (FIG. 1, *U, c, e;* FIG. 2, *C, e*) and a new hyphal connection (FIG. 1, *U, d, f;* FIG. 2, *C, f*) is formed between the parent mycelial filament and one of the three swollen cells. This secondary connection continues to serve as a passageway when in their turn the three swollen cells are progressively emptied of their protoplasm.

Thus nourished through capture and destruction of eelworms the fungus gives rise from its prostrate hyphae to scattered erect conidiophores mostly 0.3 to 0.45 mm. in height (FIG. 1, *W, a; X, a, b;* FIG. 2, *N, a*). Some hyphal segments will put forth two conidiophores, either simultaneously (FIG. 2, *O, a, b*) or successively (FIG. 1, *X, a, c*). Each conidiophore bears at its tip, which sometimes is slightly inflated, a single biseptate conidium (FIG. 1, *W, b;* FIG. 2, *N, b*) most closely resembling the conidia of *Dactylella*

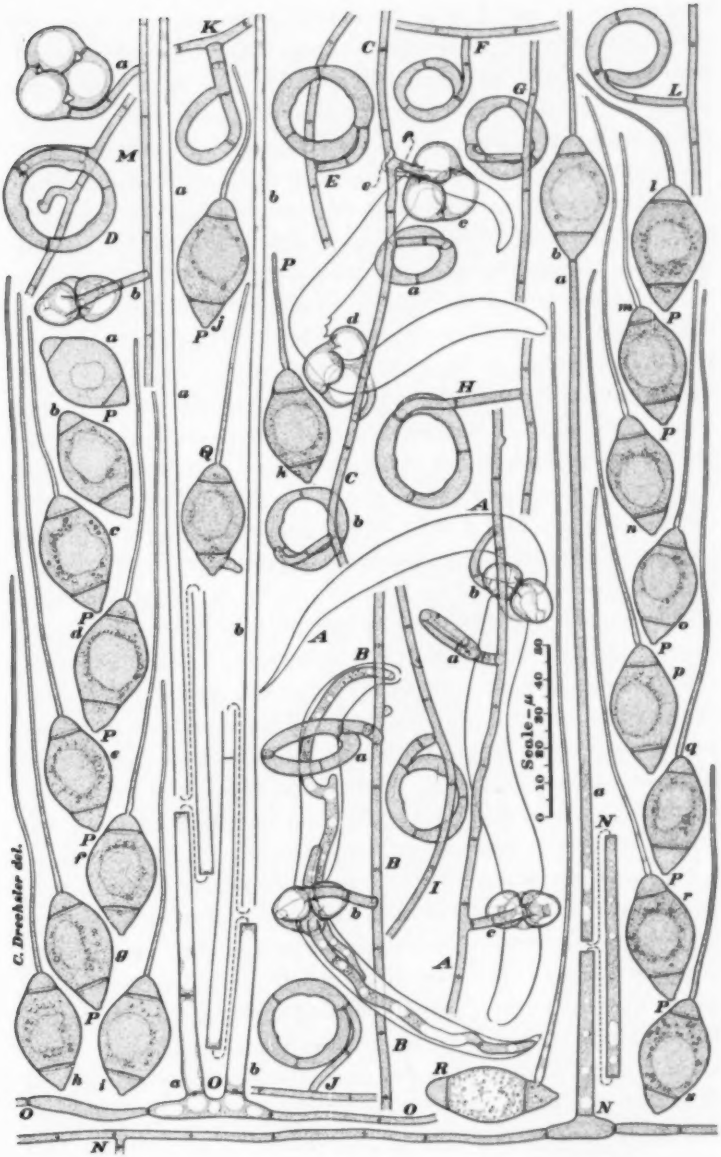

FIG. 2.

doedycoides in that it is of broadly turbinate shape and has its cross-walls placed so as to delimit a large barrel-shaped middle cell from a small basal and a small apical cell. The basal end of the detached conidium, which as a rule protrudes perceptibly from the rounded contour of the proximal segment, appears bluntly or somewhat convexly truncate in the small area of disjunction, and therefore differs rather markedly from the concave basal end in conidia of *D. doedycoides*. When sporulating tracts in nematode-infested agar plate cultures are examined under a dry objective, some of the conidia seen held aloft on conidiophores show no distal outgrowth of any kind; and such distally unmodified conidia (FIG. 1, *Y, a–f;* FIG. 2, *P, a, b*) may be found detached in moist preparations under a cover glass. However, by far the greater number of conidia produced in nematode-infested cultures have a narrow, slightly flexuous filamentous appendage that is attached at or very near the tip of the distal cell and is directed in prolongation of the spore axis (FIG. 1, *Y, g–p;* FIG. 2, *P, c–s*). Once a conidium has fallen on a moist substratum it may germinate vegetatively, it may anastomose with a mycelial hypha (FIG. 1, *S, b*), or it may put forth a filamentous outgrowth from its proximal cell (FIG. 1, *Z, a, b;* FIG. 2, *Q, R*). Although in *D. coelobrocha* conidia will often extend filamentous outgrowths liberally from positions below the large middle cell even while they are still borne aloft on their supporting hyphae, in the present fungus the individual conidium will apparently produce only its single apical appendage previous to disjunction. It seems probable that conidia lacking an apical outgrowth are still in some degree immature.

Pure cultures of the fungus are readily obtained when sterile maizemeal agar is planted with conidia removed from the tall conidiophores by means of a sterile slab of agar held on a flamed platinum spatula. The conidia germinate promptly, soon giving rise to a vegetative mycelium of radiating appearance. As in many related forms hyphal anastomoses take place abundantly. After about 5 days conidiophores and conidia begin to develop. In 15 days they are usually visible to the naked eye as a somewhat extensive downy turf. While many conidiophores produced in pure culture (FIG. 3, *A, B*) are fully as tall as those found on nematode-

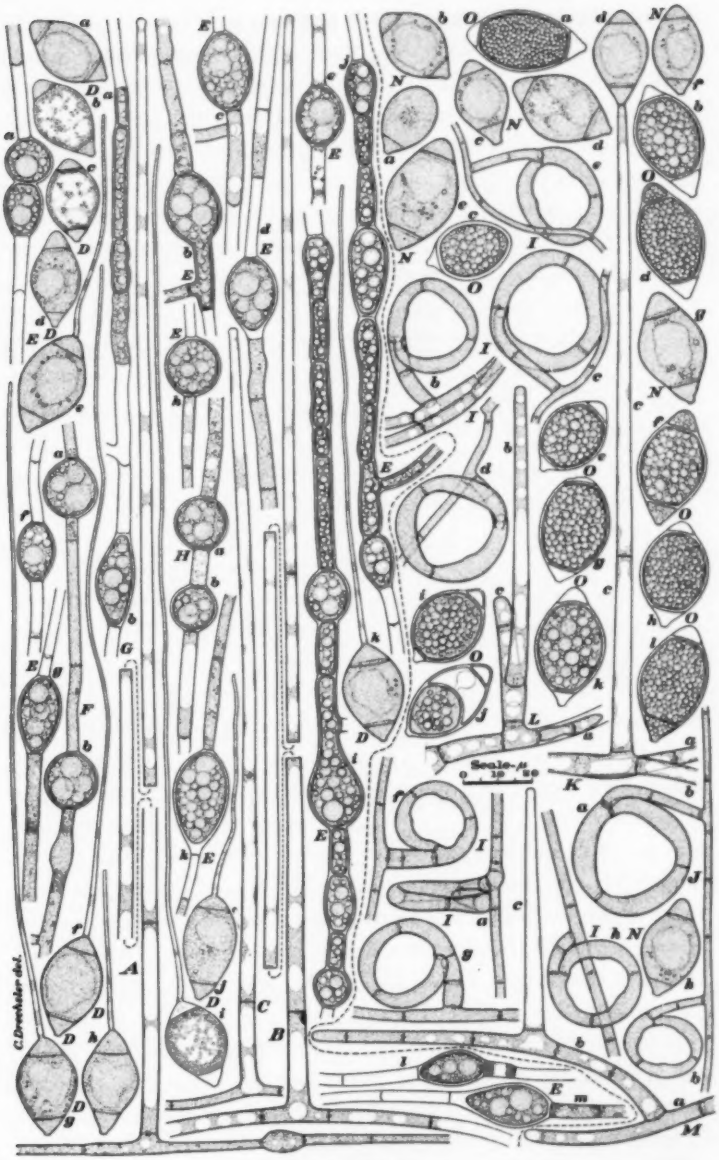

FIG. 3.

infested substratum, many others measure only 0.2 to 0.3 mm. in height, and thus may be considered of less than normal stature (FIG. 3, *C*). A similar tendency toward smaller dimensions is noticeable also in the conidia formed in pure culture (FIG. 3, *D*, *a–k*), yet numerous specimens here have approximately the same dimensions and cellular make-up as conidia nourished through destruction of eelworms. Some conidia have no filamentous outgrowth (FIG. 3, *D*, *a–d*), though usually the larger number are provided with an apical appendage of moderate (FIG. 3, *D*, *e*, *h*, *j*, *k*) or of impressive length (FIG. 3, *D*, *f*, *g*, *i*). Conidia in pure cultures 15 days old often begin to show internal modification. The less transparent contents of the broad middle segment become disposed in a thin parietal layer of granular or minutely globuliferous texture (FIG. 3, *D*, *b*, *c*). In some conidia the basal and apical segments undergo similar internal reorganization, but in other conidia either or both of these smaller cells suffer gradual loss of all their protoplasm (FIG. 3, *D*, *i*).

Meanwhile, more pronounced changes in texture of cellular contents have been proceeding in the subjacent mycelium, with resultant development of numerous chlamydospores (FIG. 3, *E*, *a–m*). These durable reproductive bodies are frequently composed, as in the very familiar *Arthrobotrys oligospora* Fres., of one or two somewhat thick-walled, distended intercalary cells densely filled with globuliferous contents (FIG. 3, *E*, *a–h*, *k–m*), but frequently, again, they consist of a more numerous chain of indurated hyphal segments (FIG. 3, *E*, *i*, *j*). In the longer chains some segments may be of cylindrical shape and only slightly wider than the unmodified portions of the parent hypha. Chlamydospores are sometimes separated from their nearest neighbors in the same mycelial filament by ordinary vegetative hyphal cells (FIG. 3, *F*, *a*, *b*), sometimes by empty cells (FIG. 3, *G*, *a*, *b*), and sometimes by one or more slightly indurated cells (FIG. 3, *H*, *a*, *b*). When fully differentiated they have a deeper yellowish coloration than the usually rather extensive complexes of inflated globuliferous cells produced in pure cultures of *Dactylella heterospora*, and more tardily also in some pure cultures of *D. doedycoides*.

The new fungus ordinarily forms no constricting rings when it grows undisturbed on maizemeal agar without admixture of any

other organism. However, if a Petri plate culture is allowed to become well infested with mites predacious rings are produced abundantly (FIG. 3, *I, a–h; J, a, b*) though in the absence of nematodes these organs have no scope for profitable functioning. Conidiophores formed in mite-infested cultures (FIG. 3, *K, c; L, b; M, c*) are conspicuously shorter than those produced in nematode-infested cultures, their height commonly varying between 75 and 200 μ. After falling over on moist substratum they give rise freely to secondary and tertiary conidiophores (FIG. 3, *K–M, a–c*). The conidia borne on them (FIG. 3, *K, d; N, a–h*) seem somewhat more often undersized and deficient in normal septation than conidia produced on nematode-infested material. They very commonly lack the distal appendage so frequently present in pure cultures and in nematode-infested ones. In many instances they are converted largely or wholly into chlamydospores (FIG. 3, *O, a–l*). Most often the large middle cell alone undergoes thickening of its wall and becomes densely filled with globuliferous contents as the smaller basal and terminal cells are emptied of protoplasm (FIG. 3, *O, a, b, e, g, h, i, k*). Sometimes either the basal cell (FIG. 3, *O, l*) or the apical cell (FIG. 3, *O, c, d*) is included with the middle cell to form a bilocular chlamydospore. Occasionally all three segments of the conidium are included in the chlamydospore (FIG. 3, *N, f*), though the middle cell then, as a rule, shows more pronounced induration than the end cells. Somewhat rarely only an upper or a lower portion of the middle cell together perhaps with the adjoining end cell (FIG. 3, *O, j*) is found indurated.

A term compounded of two words, ἄκρα and χαίτη, meaning "tip" and "hair," respectively, may serve as an appropriate epithet for the fungus.

Dactylella acrochaeta sp. nov.

Mycelium sparsum; hyphis hyalinis, ramosis, mediocriter septatis, plerumque 1.8–4.5 μ crassis, hic illic ex ramulis bilocularibus (raro trilocularibus) 12–38 μ (vulgo 18–28 μ) longis 1.8–6μ (plerumque 2–3.5 μ) crassis laqueos circulares 18–35 μ latos proferentibus qui in 3 cellulis arcuatis consistunt et foramen rotundum vel rotundo-triangulum vel rotundo-trifoveolatum 11–25 μ latum circumdant; cellulis arcuatis 15–30 μ longis, extremo 2.5–4.5 μ crassis, medio 4–7 μ crassis, saepius medio in latere foramen juxta gibbo praeditis; vermiculo nematoideo in laqueum apertum errato omnibus tribus cellulis

abrupte se contrahentibus, animal magnopere comprimentibus, id ita necantibus, integumentum ejus perforantibus, hyphas intus evolventibus quae carnem exhauriunt; hyphis assumentibus mox mediocriter septatis, saepius aliquid ramosis, maximam partem 2.3–4 μ crassis. Hyphae fertiles incoloratae, erectae, 75–475 μ (vulgo 325–450 μ) altae, basi 4–6.5 μ crassae, sursum leniter attenuatae, apice vulgo 2–2.5 μ crassae, interdum leviter tuberantes, ibi unum conidium ferentes; conidiis incoloratis, plerumque late fusiformibus, apice appendice filiformi praeditis, sine appendice vulgo biseptatis, plerumque 30–42 μ (saepius circa 35.3 μ) longis, 13.2–22.6 μ (saepius circa 19.5 μ) crassis, cellula infima eorum plerumque 5–9.2 μ (saepius circa 7.1 μ) longa, cellula media 17.2–26.2 μ (saepius circa 23 μ) longa, cellula summa 4–8.8 μ (saepius circa 5.2 μ) longa; appendice aliquid flexuosa, 25–250 μ (vulgo 50–150 μ) longa, basi 1.2–2 μ crassa, sursum 0.6–0.8 μ crassa. Chlamydosporae flavidae, valde globuliferae, quandoque ex 1–12 cellulis hyphae mycelii aliis globosis et usque 18 μ crassis aliis cylindraceis et 4–6 μ latis constantes, quandoque in 1–3 cellulis conidii praecipue in cellula conidii media consistentes.

Vermiculos nematoideos varios capiens consumensque habitat in humo silvestri prope Cumberland, Maryland.

Mycelium scanty, spreading; vegetative hyphae colorless, branched at moderate intervals, mostly 1.8 to 4.5 μ wide, often, especially in the presence of nematodes, giving rise on curving stalks to circular rings in usually perpendicular positions; the stalks 12 to 38 μ (commonly 18 to 28 μ) long, 1.8 to 6 μ (commonly 2 to 3.5 μ) wide, usually composed of 2 approximately equal cells, but occasionally of 3 cells; the rings, measuring 18 to 35 μ in diameter and surrounding a circular or rounded triangular or rounded trifoveolate aperture 11 to 25 μ wide, being regularly composed of 3 arcuate segments; the arcuate segments 15 to 30 μ long, 2.5 to 4.5 μ wide at the ends, 4 to 7 μ wide at the middle, very often having a protuberance in the middle on the concave side bordering the aperture—the first and third segments being united to one another as well as to the end of the stalk; on entrance of a nematode into the aperture the arcuate cells contracting abruptly, all three indenting the animal broadly and deeply, thereby strongly constricting it and soon disabling it, then perforating its integument and extending through its body assimilative hyphae mostly 2.3 to 4 μ wide, which appropriate the fleshy contents. Conidiophores colorless, erect, 75 to 475 μ (mostly 325 to 450 μ) high, 4 to 6.5 μ wide at the base, tapering gradually upward, 2 to 2.5 μ wide distally though sometimes slightly inflated at the tip, there bearing a single conidium. Conidia colorless, usually broadly spindle-shaped, bearing distally an appendage in approximate alignment with the spore axis, measuring (exclusive of the appendage) 30 to 42 μ (average 35.3 μ) in length and 13.2 to 22.6 μ (average 19.5 μ) in greatest width, commonly divided by 2 cross-walls into a small basal cell 5 to 9.2 μ (average 7.1 μ) long, a large barrel-shaped middle cell

17.2 to 26.2 μ (average 23 μ) long, and a small apical cell 4 to 8.8 μ (average 5.2 μ) long; the appendage colorless, filamentous, somewhat flexuous, continuous or meagerly septate, eventually empty or nearly empty of protoplasm, 25 to 250 μ (commonly 50 to 150 μ) long, 1.2 to 2 μ wide at the base, 0.6 to 0.8 μ wide toward the tip. Chlamydospores yellowish, filled with globuliferous contents, formed through induration of 1 to 12 (rarely more) hyphal cells or of 1 to 3 conidial cells; the indurated hyphal cells sometimes of cylindrical shape and measuring 7 to 35 μ in length and 4 to 6 μ in width, sometimes of subspherical or prolate ellipsoidal shape and measuring 10 to 18 μ in greatest width, but most often of intermediate shapes and dimensions; the chlamydospores of conidial origin consisting of the indurated middle cell together sometimes with either end cell or more rarely with both end cells.

Capturing and consuming different species of nematodes it occurs in leaf mold in deciduous woods near Cumberland, Maryland.

ACTIVITY OF DACTYLELLA HETEROSPORA IN DECAYING GRASS REFUSE DURING WINTER

Dactylella heterospora was first obtained, though only in small quantity, from decaying grass leaves taken from a heap of lawn clippings in Arlington, Va., early in May, 1941. In my cultures other kinds of decaying plant detritus did not yield this easily recognizable fungus during the preceding 8 years or during the ensuing 10 years. The species developed in unexpected abundance in dozens of maizemeal-agar plate cultures planted with decaying grass taken at different times during January and February, 1950, from old weed piles near Beltsville, Md., and from heaps of lawn clippings in College Park, Md. Judging from the fragments of inflorescences found mixed with disintegrating leaves and culms, the weed piles contained mainly crab-grass (*Digitaria sanguinalis* (L.) Scop.), goose-grass (*Eleusine indica* (L.) Gaertn.), foxtail (*Setaria* sp.), and Bermuda grass (*Cynodon dactylon* (L.) Pers.), while the lawn clippings included chiefly Kentucky bluegrass (*Poa pratensis* L.) and crab-grass. As some portions of the decaying grass were badly infested with mites, many of the cultures were ruined within 15 or 20 days after they had been planted, but even in these short-lived cultures *D. heterospora* often developed extensively. Its generally prompt development indicated strongly that the fungus was present in the decaying material in an active state,

and that, consequently, it was operative in the destruction of nematodes during the longer periods of mild winter weather.

The conidiophores (FIG. 4, *A*) arising from prostrate hyphae in the cultures prepared with Maryland material, as well as the characteristically biseptate conidia (FIG. 4, *A, b; B–J*) borne on them, showed no noteworthy departure from the description based on

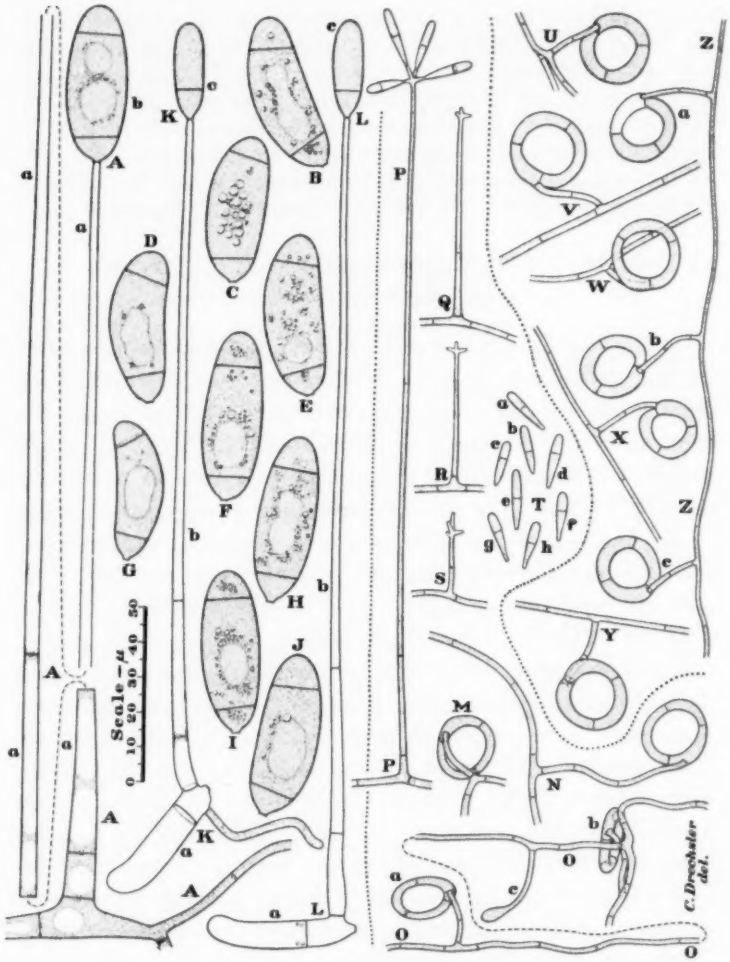

FIG. 4.

the Virginia fungus. Some of the nematode-infested cultures that were free of mites permitted repetitional development of conidia over a period of nearly 2 months. The secondary conidia formed on conidiophores sent up from fallen primary conidia were usually biseptate. Conidia of the third order (FIG. 4, *K, a; L, a*) appeared very frequently to be divided by only one septum, and when in their turn they gave rise individually to an erect conidiophore (FIG. 4, *K, b; L, b*) the small conidia of the fourth order (FIG. 4, *K, c; L, c*) likewise had only one cross-wall. The reduced septation and size made for closer similarity to the somewhat allantoid, regularly uniseptate conidia that in pure culture are formed on short rachiform, procumbent hyphal branches.

AN ARTHROBOTRYS-LIKE HYPHOMYCETE CAPTURING EELWORMS IN DELICATE NON-CONSTRICTING RINGS

A maizemeal-agar plate culture which after being overgrown with mycelium of *Pythium irregulare* Buisman had been further planted with a small quantity of leaf mold taken from deciduous woods near Chester, Pa., on Feb. 14, 1950, showed 15 days later a delicate hyphomycete subsisting evidently through capture of nematodes by means of 3-celled non-constricting rings very similar to those earlier observed in my *Dactylella lysipaga* (**2**: 499–504), in my *Dactylella leptospora* (**2**: 504–508), in *Dactylaria candida* (Nees) Saccardo (**2**: 523–527), and in *Anulosporium nematogenum* Sherbakoff (**12**). The rings commonly measured 18 to 20 μ in diameter and inclosed an aperture 12 to 14 μ wide. Although occasionally supported on a 1-celled (FIG. 4, *M*) or 3-celled (FIG. 4, *N*) stalk, they were more usually borne on a 2-celled stalk (FIG. 4, *O, a, b*) measuring 15 to 25 μ in length and about 1.7 μ in width. The rather scanty mycelium, which occupied an area not exceeding 25 square millimeters, revealed no stalked adhesive knobs of the type accompanying similar predacious rings in other species. In an early stage of development the ring, much as in related forms, had somewhat the appearance of a stalked knob (FIG. 4, *O, c*).

The delicate mycelium gave rise from procumbent hyphae to sparsely scattered, erect or ascending, colorless conidiophores (FIG. 4, *P–S*) which measured 25 to 200 μ in length, 1.5 to 2.3 μ in basal

diameter and 1 to 1.2 μ in apical width. Near the tip these conidiophores commonly gave off 2 or 3 lateral spurs usually measuring 2 to 3 μ in length. Each spur as well as the axial tip bore a conidium, so that 3 or 4 conidia were held aloft in loose capitate arrangement (FIG. 4, *P*). The conidia (FIG. 4, *T*, *a–h*) were colorless, uniseptate, and of generally clavate shape. They measured mostly 13 to 17 μ in length and 1.5 to 2 μ in greatest width. Their single cross-wall was more often placed a little above the middle (FIG. 4, *T*, *a*, *c*, *d*, *f*, *g*, *h*) than at the middle (FIG. 4, *T*, *e*) or below the middle (FIG. 4, *T*, *b*). Many of the taller conidiophores, though at first erect, later gradually sank into a reclining position, and would then frequently put forth much shorter secondary conidiophores.

My attempts to isolate the fungus by removing its conidia to sterile agar were all unsuccessful, owing to the unfortunate circumstances that the area occupied by it was occupied also by several other predacious hyphomycetes with more robust and taller conidiophores. Consequently I was unable to determine whether the delicate *Arthrobotrys*-like conidial apparatus represented a main asexual reproductive phase like that of my *A. entomopaga* (**8**) or a subsidiary sporulating stage comparable to the secondary conidial stage found in my *Dactylaria psychrophila* (**7**) and my *Dactylaria cudermata* (**10**). Similar difficulty was encountered earlier in a nematode-capturing hyphomycete that employed short-stalked adhesive knobs as predacious organs and gave rise to conidial apparatus best referable to *Trichothecium* (**7**: 166–168).

ANOTHER FUNGUS ENSNARING NEMATODES IN THICKISH NON-CONSTRICTING RINGS

A maizemeal-agar plate culture which after being overgrown by *Pythium spinosum* Sawada had been further planted with a pinch of leaf mold taken from deciduous woods near Cumberland, Md., on Feb. 6, 1951, showed on examination 38 days later a somewhat extensive mycelium that subsisted evidently through capture of nematodes in non-constricting rings (FIG. 4, *U–Y*; *Z*, *a–c*). These rings, borne on 2-celled stalks mostly 13 to 20 μ long and 1.2 to 2.1 μ wide, measured 17 to 22 μ in outside diameter and had a

round aperture 9.5 to 15 μ wide. The three arcuate cells composing them varied in width between 2.8 and 4.8 μ, and thus were noticeably stouter than the corresponding cells in *Dactylella lysipaga, Dactylella leptospora* and *Dactylaria candida*. In their more massive conformation the predacious organs here resembled the stout non-constricting rings observed earlier (**10**: 58–60) in agar plate cultures planted with leaf mold from Greensboro, N. C. With respect to the outside diameter of its predacious rings, however, the fungus from western Maryland agreed much more closely with the three nematode-ensnaring species to which binomials have been given than with the unnamed form occurring in North Carolina.

ANOTHER SMALL TRICHOTHECIUM PARASITIZING PYTHIUM OOSPORES

In earlier papers (**3, 5**) *Trinacrium subtile* Riess and two species of hyphomycetes that I described under the binomials *Dactylella spermatophaga* and *Trichothecium arrhenopum* were set forth as developing parasitically on *Pythium oospores*. Despite their commonplace appearance these fungi are of considerable interest not only because of their efficacy in destroying the resting bodies by which important plant pathogens live over in soil from year to year, but also because of their apparently close taxonomic relationship to the clampless nematode-capturing hyphomycetes. Another species of similar destructive capacity and similar kinship came to light in some maizemeal-agar plate cultures which after being overgrown by *Pythium spinosum* had been further planted with a small quantity of leaf mold taken up near Hermiston, Ore., on Aug. 20, 1947. Numerous oospores of *P. spinosum* were found occupied by relatively massive assimilative branches coming from a delicate septate mycelium (FIG. 5, *A–C*) that permeated the agar sparingly yet extensively. Often an expanded portion of hypha, or appressorium, was broadly in contact with the oogonial wall where this wall had been penetrated (FIG. 5, *B, C*) but sometimes no pronounced external enlargement of the invading hypha could be observed (FIG. 5, *A*). Wherever external appressoria could be recognized, they were generally found in nearly straight alignment with their supporting hyphae, and thus did not show the curious

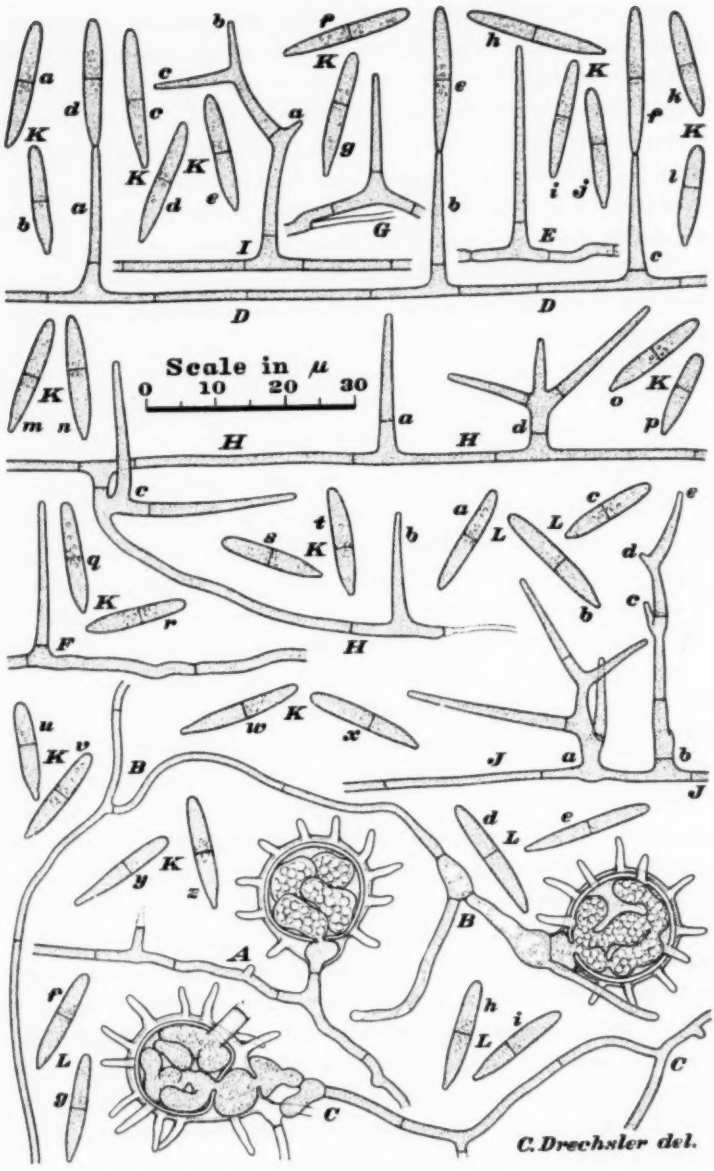

FIG. 5.

crook-necked antheridium-like conformation usual in appressoria of *Trichothecum arrhenopum*. Intimate enwrapment of the oogonium by the hypha bearing the appressorium, a characteristic feature in the parasitism of *Trichothecium arrhenopum*, was wholly absent in the Oregon fungus. In the space between the oogonial envelope and the oospore the invading hypha often showed an enlargement, which very probably represented an appressorium that had served in penetrating the oospore wall. The massive, somewhat lobulated assimilative branches contained globuliferous protoplasm while the host contents were being absorbed, but later this protoplasm was gradually withdrawn backward into the external mycelium, until in the end only the empty hyphal membrane remained within the empty envelopes of oospore and oogonium.

In the same agar plate cultures the Oregon fungus was found also parasitizing scattered individuals of the testaceous rhizopod *Arcella vulgaris* Ehrenb. and clustered cysts of a species of *Polyangium*. Although parasitism of fungi on bacteria might seem an unlikely biological relationship, cysts of Myxobacteriales produced on or under the surface of agar substratum have so often been found invaded by hyphomycetes of the predacious series that in nature they must almost certainly suffer widespread destruction from such attack. Invasion of *A. vulgaris* by the present fungus is hardly to be considered anomalous, since related hyphomycetes similarly subsisting for the most part on oospores will likewise destroy many individuals of suitable testaceous rhizopods encountered by them. Conversely, my *Dactylella passalopaga* (**1**) and other hyphomycetes subsisting mainly through capture of testaceous rhizopods are often abundantly destructive to oospores of such common damping-off parasites as *Pythium debaryanum*, *P. ultimum* Trow, and *P. irregulare*.

The fungus from Oregon gave rise at intervals along its procumbent hyphae to erect conidiophores (FIG. 5, *D*, *a–c*; *E–G*; *H*, *a*, *b*) generally somewhat shorter than those of *Trichothecium arrhenopum*. On each conidiophore a single conidium was produced (FIG. 5, *D*, *d–f*). Many conidiophores, in addition, gave off one (FIG. 5, *H*, *c*) or two (FIG. 5, *H*, *d*) or three (FIG. 5, *J*, *a*) branches, each of which likewise bore a terminal conidium. In

some ramified conidiophores (FIG. 5, *I; J, b*) the several sporiferous parts (FIG. 5, *I, a–c; J, c–e*) evidently were formed through successive branching. The conidia (FIG. 5, *K, a–z; L, a–i*) seemed generally shorter and narrower than those of *T. arrhenopum*, which in most other respects they resembled rather closely.

As its more commonplace manner of attacking oospores makes the fungus easily distinguishable from *Trichothecium arrhenopum* it is described as a separate species. Its assignment to the form genus typified by *T. roseum* Link is not intended to imply any intimate taxonomic kinship with that very familiar mold. The wide scope of its parasitism may perhaps be helpfully suggested in an epithet meaning "much slaying."

Trichothecium polyctonum sp. nov.

Mycelium ramosum, saepe aliquid sparsum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 1.2–1.8 μ crassis, hic illic appressoria clavata vulgo 4–6 μ crassa ad oosporas Pythii et rhizopoda crustosa et corpora perdurantia myxobacteriacearum apponentibus, murum perforantibus, hyphas assumentes lobosas 3–5 μ crassas intrudentibus; hyphis fertilibus erectis, incoloratis, primo simplicibus, vulgo pauce septatis, saepius 15–30 μ altis, basi 2–3 μ crassis, apice 0.6–1 μ crassis, unum conidium ferentibus, postea saepe aliquid ramosis denique 1–3 alia conidia gerentibus; conidiis incoloratis, clavulatis vel elongato-ellipticis, basi saepius aliquid attenuatis et abrupte truncatis, apice rotundatis, medio uniseptatis, plerumque 12.7–21 μ (saepe circa 16.9 μ) longis, 2.3–2.8 μ (saepe circa 2.6 μ) crassis.

Arcellam vulgarem et oosporas *Pythii spinosi* et corpora perdurantia *Polyangii* enecans habitat in humo silvestri prope Hermiston, Oregon.

Mycelium branched, often rather scanty. Vegetative hyphae colorless, septate at moderate intervals, mostly 1.2 to 1.8 μ wide, on encountering suitable hosts often forming clavate appressoria 4 to 6 μ wide, then narrowly perforating the host wall and intruding lobulated assimilative branches 3 to 5 μ wide. Conidiophores erect, colorless, at first unbranched, meagerly septate, often with only one cross-wall near the base, 15 to 30 μ high, 2 to 3 μ wide at the base, 0.6 to 1 μ wide at the tip and there bearing a single conidium, later often producing 1 to 3 branches, and on each branch bearing an additional conidium. Conidia colorless, somewhat clavate or of elongate elliptical outline, often narrowing at the abruptly truncate base, rounded at the tip, regularly divided by a cross-wall at the middle, mostly 12.7 to 21 μ (average 16.9 μ) long and 2.3 to 2.8 μ (average 2.6 μ) wide.

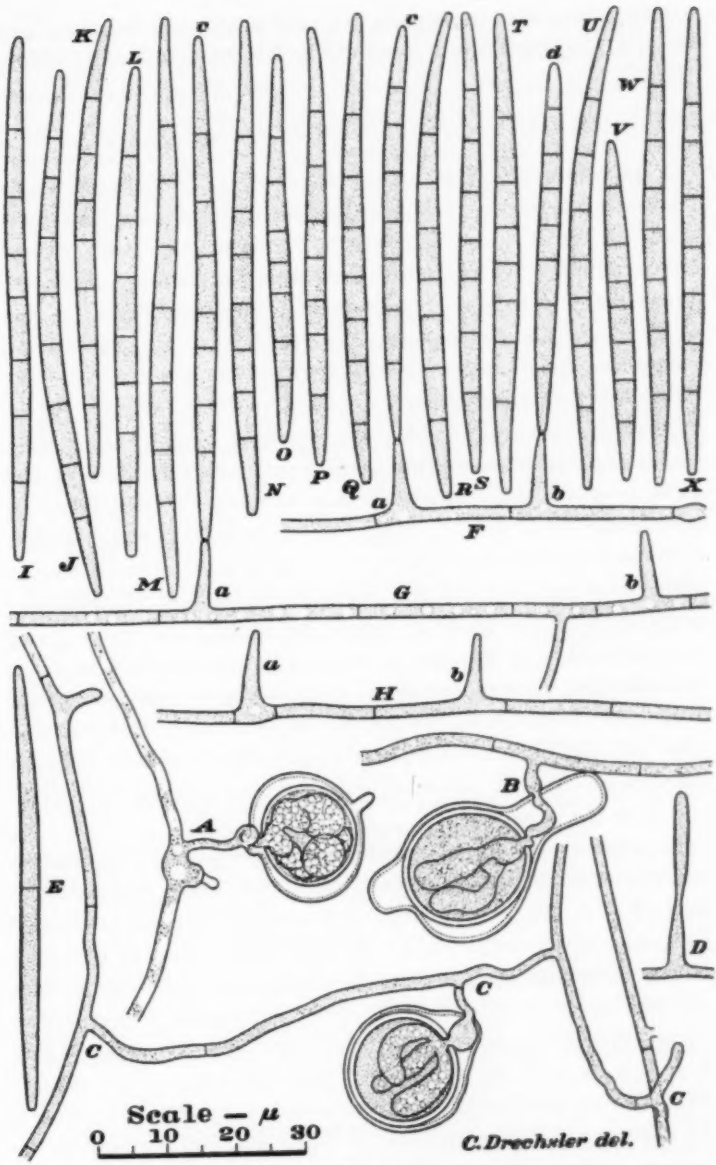
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FIG. 6.

Parasitizing *Arcella vulgaris*, oospores of *Pythium spinosum*, and cysts of *Polyangium* sp., it occurs in leaf mold near Hermiston, Oregon.

A FUSARIUM-LIKE DACTYLELLA DESTRUCTIVE TO PYTHIUM OOSPORES

A maizemeal-agar plate culture which after being overgrown by *Pythium debaryanum* had been further planted with leaf mold taken up near Provo, Utah, late in the summer of 1947, showed on examination 45 days later many oospores of the *Pythium* destroyed by a colorless septate mycelium generally similar to that of *Trichothecium polyctonum*. Penetration of the oogonial envelope was here usually accomplished with little (FIG. 6, *A, B*) or no external enlargement (FIG. 6, *C*) of the invading hypha. Within the oogonial chamber the invading hypha was in some instances modified very little before it penetrated the oospore wall (FIG. 6, *A, B*) but in other instances it showed a fairly large appressorium (FIG. 6, *C*). After the oospore wall was narrowly perforated assimilative branches of lobulate or broadly digitate shape were intruded into the protoplasmic interior. Gradual appropriation of the spore contents and withdrawal of all living material backward into the external mycelium followed much as in destruction of oospores by *T. polyctonum*. Zygospores of the zoopagaceous parasite *Cochlonema megalosomum* Drechsl. that were grouped here and there in the agar culture around collapsed pellicles of *Amoeba verrucosa* Ehrenb. were attacked and parasitized in the same way.

In initiating asexual reproduction prostrate hyphae of the fungus extended erect branches noticeably constricted about 10 μ above the base (FIG. 6, *D*). A cross-wall at the constriction soon delimited the longer distal portion as a conidium, which after being divided first by a median septum (FIG. 6, *E*) usually underwent further partitioning into 8 subequal cells. The procumbent filaments thus came eventually to support at intervals relatively short spurs (FIG. 6, *F, a, b; G, a, b; H, a, b*) each of which bore a slender 7-septate (FIG. 6, *F, c, d; G, c; I; K–X*) or occasionally 8-septate (FIG. 6, *J*) conidium rather suggestive of *Fusarium*, though lacking the curious basal modification frequent in that genus. In view of the

equally perplexing resemblance to *Fusarium* that is evident in conidia more particularly of the nematode-capturing *Dactylella leptospora* and the rhizopod-capturing *D. passalopaga*, the Utah fungus would seem, despite its ambiguous appearance, to belong taxonomically with the clampless predacious hyphomycetes. The epithet, meaning "like a worm," that is here applied to it, is intended to have reference to the conformation of its spores.

Dactylella helminthodes sp. nov.

Mycelium sparsum, ramosum; hyphis sterilibus incoloratis, mediocriter septatis, plerumque 1–2.5 μ crassis, hic illic ramulos assumentes inflatos vel lobosos et vulgo 3–6 μ crassos in sporas perdurantes intrudentibus; hyphis fertilibus incoloratis, erectis, plerumque circa 10 μ altis, basi saepius 2–3 μ crassis, sursum attenuatis, apice circa 1 μ crassis, ibi unum conidium ferentibus; conidiis hyalinis, elongato-fusoideis, basi rotundo-truncatis, apice rotundatis, vulgo 7-septatis, rarius 8-septatis, plerumque 53–84 μ (saepe circa 67.3 μ) longis, 2.8–3.7 μ (saepe circa 3.1 μ) crassis.

Oosporas *Pythii debaryani* et zygosporas *Cochlonematis megalosomi* enecans habitat in humo silvestri prope Provo, Utah.

Mycelium sparse, branched; vegetative hyphae colorless, septate at moderate intervals, mostly 1 to 2.5 μ wide, here and there intruding into indurated resting spores inflated or lobulated assimilative branches commonly 3 to 6 μ wide; conidiophores colorless, erect, mostly about 10 μ high, 2 to 3 μ wide at the base, tapering upward, at the tip about 1 μ wide and there bearing a single conidium; conidia colorless, elongated fusiform, somewhat convexly truncate at the base, rounded at the tip, usually 7-septate and occasionally 8-septate, measuring mostly 53 to 84 μ (average 67.3 μ) in length and 2.8 to 3.7 μ (average 3.1 μ) in greatest width.

Parasitic on oospores of *Pythium debaryanum* and on zygospores of *Cochlonema megalosomum* it occurs in leaf mold near Provo, Utah.

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EXPLANATION OF FIGURES

FIG. 1. *Dactylella acrochaeta*, drawn at a uniform magnification with the aid of a camera lucida from nematode-infested agar plate cultures; × 500 throughout. *A*, Portion of hypha with a constricting ring in normal position. *B*, Portion of hypha with one ring, *a*, in normal position and another, *b*, turned flatwise. *C–E*, Portions of hyphae, each with a ring in oblique position. *F–H*, Portions of hyphae, each with a ring turned flatwise. *I*, Portion of hypha with two rings, *a* and *b*, turned flatwise. *J–R*, Portions of hyphae, each with a ring turned flatwise. *S*, Mycelial hypha that bears a closed ring, *a*, on an unusually long stalk and that has anastomosed with a conidium, *b*. *T*, Portion of mycelium with two rings, *a* and *b*, in early stages of development; with two rings, *c* and *d*, that have closed emptily; and with one ring, *e*, that has killed and invaded an eelworm possibly referable to *Plectus* sp. *U*, Hypha with two rings, *a* and *b*, that have captured an eelworm; the stalk *c* of ring *a* having degenerated, a secondary connection, *d*, has been formed; the stalk *e* of ring *b* having also degenerated has been replaced by a secondary connection, *f*. *V*, Portion of mycelium bearing a ring on its original stalk; the ring has closed on an eelworm and extended assimilative branches into it. *W*, Portion of prostrate hypha with an erect conidiophore, *a*, bearing a conidium, *b*. *X*, Portion of prostrate hypha with two erect denuded conidiophores, *a* and *b*, and with a third, *c*, in very early stage of growth. *Y*, Conidia; some of them, *a–f*, without appendages; others, *g–p*, having each an apical appendage. *Z*, Conidia, *a* and *b*, that after falling on moist substratum are extending an outgrowth from the basal cell. (Owing to lack of space the conidiophores *W*, *a*, and *X*, *a*, are shown in parts whose proper connection is indicated by broken lines.)

FIG. 2. *Dactylella acrochaeta*, drawn at a uniform magnification with the aid of a camera lucida from nematode-infested agar plate cultures; × 500 throughout. *A*, Portion of hypha with an open ring, *a*, in normal vertical position, and two closed rings, *b* and *c*, that have captured an eelworm. *B*, Portion of hypha with an open ring, *a*, turned slightly sideways, and a closed

ring, *b*, that has caught an eelworm and extended assimilative hyphae into it. *C*, Portion of hypha with two open rings, *a* and *b*, turned flatwise, and two closed rings, *c* and *d*, that have jointly captured a nematode; the stalk *e* of ring *c* having degenerated, a new connection, *f*, has been formed. *D–J*, Portions of hyphae, each with a ring turned flatwise. *K*, *L*, Incompletely developed rings. *M*, Portion of hypha with one closed ring, *a*, turned flatwise and with another, *b*, in normal position. *N*, Prostrate hypha with an erect conidiophore, *a*, bearing a conidium, *b*. *O*, Prostrate hypha with two denuded conidiophores, *a* and *b*. *P*, Conidia; some of them, *a* and *b*, without an appendage; others, *c–s*, having each an apical appendage. *Q*, *R*, Conidia, each with an outgrowth arising from its basal cell. (Owing to lack of space the conidiophores *N*, *a*, and *O*, *a* and *b*, are shown in parts whose proper connection is indicated by broken lines.)

FIG. 3. *Dactylella acrochacta*, drawn at a uniform magnification with the aid of a camera lucida; × 500 throughout. *A–H*, Parts developed in pure culture on maizemeal-agar plates: *A–C*, Portions of prostrate hyphae, each with a denuded conidiophore; owing to lack of space the conidiophores in *A* and *B* are shown in parts whose proper connection is indicated by broken lines. *D*, Conidia; some, *a–d*, without an appendage; others, *e–k*, bearing an apical appendage. *E*, Chlamydospores, *a–m*, consisting of a single indurated segment or of several such segments in a continuous series. *F–H*, Portions of hyphae in which two chlamydospores, *a* and *b*, are separated by one or more segments. *I–O*, Parts developed in maizemeal-agar plate cultures 40 to 45 days old and well infested with mites: *I*, Portions of hyphae, each with an open ring; one of the rings, *a*, shown in normal position; the others, *b–h*, turned flatwise. *J*, Portion of hypha with two open rings, *a* and *b*, turned flatwise. *K*, Portion of a primary conidiophore, *a*, with proximal portion of a secondary conidiophore, *b*, from which arises a tertiary conidiophore, *c*, bearing a conidium, *d*. *L*, Portion of a prostrate conidiophore, *a*, from which has arisen the denuded secondary conidiophore *b* that has begun putting forth a tertiary conidiophore, *c*. *M*, Distal portion of a prostrate primary conidiophore, *a*, from which was extended the secondary conidiophore *b* that supports a denuded tertiary conidiophore, *c*. *N*, Conidia, *a–h*, without any appendage. *O*, Conidia, *a–l*, partly or wholly converted into chlamydospores.

FIG. 4. Drawn at a uniform magnification with the aid of a camera lucida; × 500 throughout. *A–L*, *Dactylella heterospora: A*, Portion of mycelium with a conidiophore, *a*, bearing a conidium, *b*. *B–J*, Conidia that were produced on conidiophores arising from prostrate hyphae. *K–L*, Conidia apparently of third order, *a*, each with an erect germ conidiophore, *b*, on which is borne a conidium of the fourth order, *c*. *M–T*, Unnamed *Arthrobotrys*-like nematode-capturing hyphomycete: *M*, *N*, Portions of mycelial hyphae, each with a stalked non-constricting ring. *O*, Portion of mycelium with two completed rings, *a* and *b*, and a third, *c*, in an early stage of development. *P*, Tall conidiophore bearing four conidia. *Q–S*, Shorter conidiophores in denuded state. *T*, Detached conidia, *a–h*, showing variations in shape and position of cross-wall. *U–Z*, Unnamed nematode-capturing hyphomycete with thickish non-constricting rings: *U–Y*, Portions of hyphae, each bearing a ring. *Z*, Longer portion of hypha bearing three rings, *a–c*. (Owing to

lack of space the conidiophore *A*, *a*, and the mycelium *O* are shown in parts whose proper connection is indicated by broken lines.)

FIG. 5. *Trichothecium polyctonum*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–C*, Portions of mycelium, each showing invasion of an oospore of *Pythium spinosum*. *D*, Portion of hypha with three simple conidiophores, *a–c*, bearing the conidia *d–f*, respectively. *E–G*, Portions of hyphae, each with a denuded simple conidiophore. *H*, Portion of hypha with two simple conidiophores, *a* and *b*, and two branched conidiophores, *c* and *d*. *I*, Portion of hypha bearing a branched conidiophore with three successively formed tips, *a–c*. *J*, Portion of hypha with one ramified conidiophore, *a*, that shows no obvious sequence of branching, and another conidiophore, *b*, that shows successive subapical branching in forming its three denuded tips, *c–e*. *K* (*a–z*), *L* (*a–i*), Detached conidia showing variations in size and shape.

FIG. 6. *Dactylella helminthodes*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A–C*, Portions of mycelium, each showing invasion of an oospore of *Pythium debaryanum*. *D*, Portion of prostrate hypha with a young conidiiferous branch. *E*, Detached immature conidium divided only by a single cross-wall. *F*, Portion of hypha with two conidiophores, *a* and *b*, bearing the conidia *c* and *d*, respectively. *G*, Portion of mycelium with two conidiophores *a* and *b*, the former bearing a conidium, *c*. *H*, Portion of hypha with two denuded conidiophores, *a* and *b*. *I–X*, Detached conidia showing usual variations in shape and size, all containing seven cross-walls except *J*, which contains eight.

TWO NEW ASCOMYCETES ON PHORADENDRON

ANNA E. JENKINS AND DONALD P. LIMBER [1]

(WITH 2 FIGURES)

A myringiale and a dothideale on *Phoradendron* are here described each as a new species. These were collected by F. A. Wellman, plant pathologist, Office of Foreign Agricultural Relations, U. S. Department of Agriculture, while stationed at the Centro Nacional de Agronomia, San Salvador, El Salvador, Central America.

Uleomyces wellmanii Jenkins & Limber, sp. nov. (FIG. 1, *A*, *a*, *B*, *C*; and FIG. 2, *A–C*)

Ascomata amphigena, praecipue hypophylla, superficialia, separata vel confluentia, in caespites subcirculares 1–6 mm. in diam. vel coalescentes majoresque aggregata, purpureo-brunnea, 180–230 μ in diam. et altitudine, loculis periphericis 24–28 × 14–21 μ praedita; asci ovoidei, bitunicati, octospori, 23–27 × 19–20 μ, tunica exteriori scindente et interiorem usque 36 μ longam et 26 μ latam extendere permittente; ascosporae hyalinae, clavatae, muriformes, transverse 3-septatae et subconstrictae, verticaliter 1-vel interdum 2-septatae, rectae vel subcurvatae, 16–19 × 7–9 μ.

In foliis *Phoradendri robustissimi*, El Salvador.

Ascomata amphigenous, chiefly hypophyllous, superficial, separate or confluent, aggregated in circular to somewhat irregular groups, 1–6 mm. in diam., larger through coalescence, in color "burnt lake" to "diamine brown," [2] individual ascomata 180–230 μ in diam., 180–230 μ high, with locules (24–28 × 14–21 μ) scattered in the peripheral region; asci ovoid, double-walled, 8-spored, 23–27 × 19–20 μ, outer ascus wall splitting, allowing inner wall to expand, reaching at least 36 μ in length and 26 μ in width; ascospores hyaline, clavate, muriform, with 3 transverse and 1, sometimes 2, vertical septae, slightly constricted at the transverse septae, straight or slightly curved, 16–19 × 7–9 μ.

[1] Thanks are extended to Julian H. Miller who verified our generic placing of the second ascomycete here described. We are indebted also to Edith K. Cash for translating the diagnoses into Latin.

[2] Ridgway, R. Color standards and color nomenclature. 43 pp. Washington, D. C.

FIG. 1. *A, B.* Leaves of *Phoradendron robustissimum* bearing ascomata of *Rosenscheldiella phoradendri* (*A*, except *a*) and of *Ulcomyces wellmanii* (*A*, *a* and *B*), × 1. *C.* Part of *B*, × 3½. *D.* Part of *A*, × 3½. *E.* Ascomata of *Ulcomyces* in section, × 500. *F.* Ascospores, × 570. *G.* Asci of *Rosenscheldiella*, × 280. *H.* An ascus, × 440. Photographs by R. L. Taylor (*A-D*) and Limber (*E-H*).

On *Phoradendron robustissimum* Eichl., growing on *Psittacanthus calyculatus* G. Don, La Cieba, El Salvador, Central America, August 20, 1943, F. L. Wellman *476*. Type in Mycol. Collect.

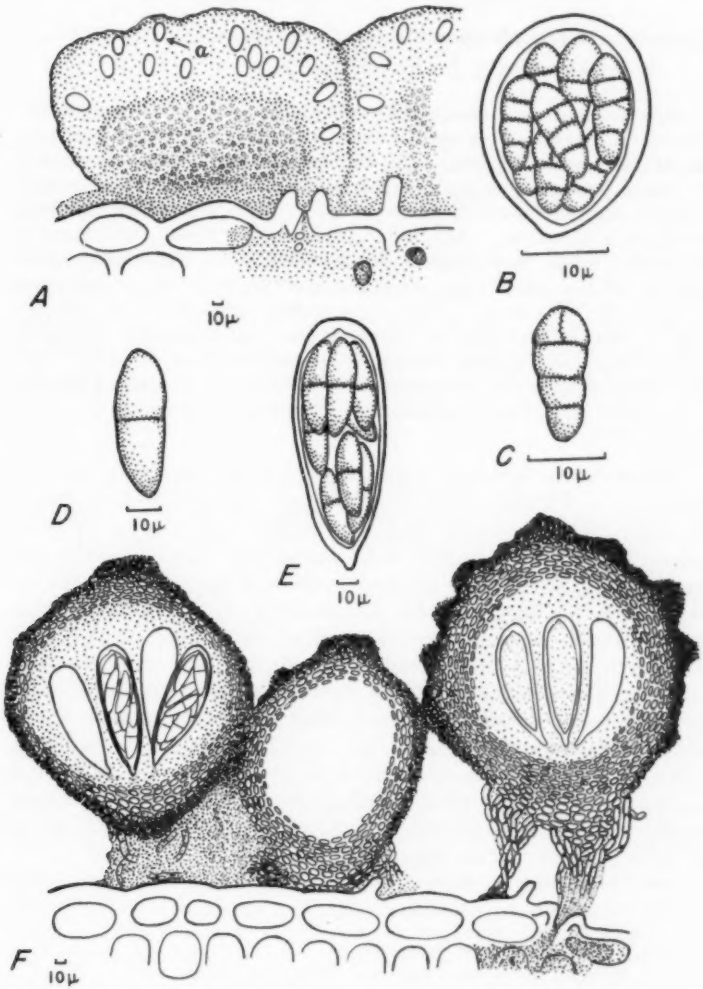

FIG. 2. *A–C. Uleomyces wellmanii. A.* Ascomata in section. *B.* Ascus. *C.* Ascospore. *D–F. Rosenscheldiella phoradendri: D.* Ascospore. *E.* Ascus. *F.* Ascomata. Drawn by D. P. Limber.

Bur. Plant Indus. Soils, and Agr. Engin. (*90433*) and Path. Herb. Bur. Ent. and Plant Quar., U. S. Dept. Agr., and Secc. Fitopat. Inst. Biol. São Paulo (*5354*), also in Jenkins-Bitancourt Myriangiales selecti exsiccati (no. *500*).

Rosenscheldiella phoradendri Limber & Jenkins, sp. nov. (FIG. 1, *A*, not including *a*, *D*; FIG. 2, *D–F*)

Stromata amphigena, praecipue hypophylla, superficialia, in caespites circulares vel irregulares 1–6 mm. in diam. vel coalescentes majoresque dense aggregata, brunneo-atra, uniloculata vel interdum loculis duobus aut pluribus in quoque stromate praedita, semiglobosa usque obconica et plus minusve angulata, stromate basali folium penetranti, 214–230 × 214–264 μ, parietibus pseudoparenchymaticis, cellulis exterioribus magnis fuscis crassi-tunicatis, interioribus parvis, tenui-tunicatis, demum diffringentibus compositis, ostiolo vero carenti, poro apicali lysigeno; asci ad basim fasciculati, late clavati, breve stipitati, 120–130 × 26–35 μ, tunicis exterioribus scindentibus et interiores crassas elasticas protrudere et sporas liberare permittentibus; ascosporae oblongo-ellipticales, utrinque obtusae, medio septatae, non constrictae, hyalinae usque viridiusculae, granulosae, 35–48 × 12.8–16 μ; paraphyses carentes.

In foliis *Phoradendri robustissimi*, *Ulcomycete wellmannii* consociata, El Salvador.

Stromata amphigenous, chiefly hypophyllous, superficial, densely cespitose, in circular to irregular groups, 1–6 mm. in diam. larger by coalescence, brownish-black, uniloculate, sometimes 2 or more locules in a stroma, semiglobose to obconic and more or less angular, basal stroma penetrating the leaf tissue and merging with the internal fungus tissue, 214–230 × 214–264 μ, with pseudoparenchymatous walls, outer parts composed of large, thick-walled dark cells, and inner, of small thin-walled cells, the latter disintegrating as the asci develop, true ostiole lacking, apical pore formed lysigenously; asci arising in a basal fascicle, broadly clavate with a short attenuated stipe, 120–130 × 26–35 μ, their walls splitting, allowing the thick, elastic inner wall to protrude, liberating the spores; ascospores oblong-elliptical, with obtuse ends, medianly septate with no constriction, hyaline to greenish, granular, 35–48 × 12.8–16 μ; no paraphyses.

On *Phoradendron robustissimum* Eichl. associated with *Ulcomyces wellmanii*, on Wellman *476*, cited above.

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A NEW SPECIES OF MUCOR [1]

EDWARD E. BUTLER [2]

(WITH 1 FIGURE)

An apparently undescribed species of *Mucor* was isolated on three occasions from diseased roots of strawberry plants during July, 1949.

This species is characterized by unusually large spores, more or less uniform in size and shape (FIG. 1, *C*), with golden yellow contents, and by an alcoholic odor in culture. Young sporangiophores are recurved (FIG. 1, *B*); older ones are tall, delicately slender and are very sensitive to white light.

Mucor recurvus sp. nov.

Herba subalba vel paene lutea vel ochreofulva, 4–5 cm. altitudine. Sporangiophoribus 8–40 μ diametro, primo recurvatis, deinde rectis, sine ramis vel cum ramis sympodialibus. Sporangiis globosis subtiliter echinulatis, ochreoluteis vel ochreocroceis (40–)70–150(–200) μ; membrana diffluenti, collarello crystallis acuformibus crustato. Columella ovali, aliquantulo pyriformi vel globosa, basi paene plana (22–)40–80(–90) × (30–)50–105(–125) μ continenti nonnumquam materiam aureofuscam vel fuscam. Sporis ovalibus vel aliquantulo reniformibus (17–)20–26(–30) × (8–)10–12(–14) μ, continentibus materiam aureoluteam. Zygosporis et gemmis non notatis.

Habitat inter radices fragi domestici (*Fragaria* spp.).

Turf grayish-white [3] to pale yellow orange to ochraceous-buff, 4–5 cm. in height. Cultures with an odor of alcoholic fermentation. Sporangiophores 8–40 microns in diameter, usually about 12–22 microns; at first recurved, later erect, then collapsing and

[1] Paper No. 2774, Scientific Journal Series article, Minnesota Agricultural Experiment Station.

[2] The author is indebted to Dr. G. A. Christenberry, Furman University, Greenville, South Carolina, for examining cultures and making helpful suggestions, and to Dr. Norman J. DeWitt, Department of Classical Languages, University of Minnesota, for preparation of the Latin description.

[3] With the exception of the terms "grayish-white," "golden brown," "brown," and "golden yellow" the terms used to describe colors are after Ridgway, 1912.

producing a more or less dense mat, unbranched or branched sympodially. Numerous short branches with small, often sterile, sporangia appear in aging cultures. Sporangia globose, finely echinulate, ochraceous orange to ochre yellow, (40–)70–150(–200)μ, with a rapidly diffluent membrane leaving a collarette (FIG. 1, *A*) encrusted with needle-shaped crystals. Columella oval (FIG. 1, *A*), somewhat pyriform or globose, with a flattened base (22–)40–80

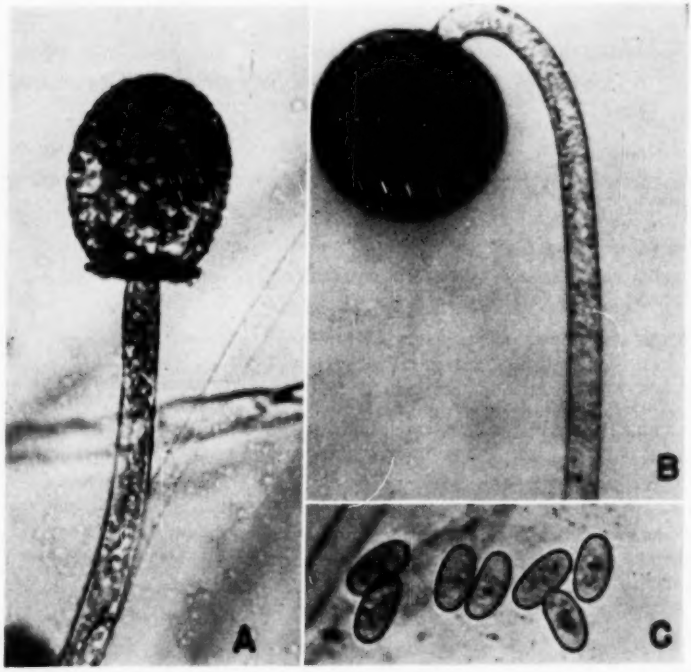

FIG. 1. *A*. Columella. Protoplasm somewhat abnormal due to killing solution, × 450. *B*. Young sporangium showing columella within, × 450. *C*. Spores, × 400.

(–90) × (80–)50–105(–125)μ with or without golden brown to brown contents. Spores oval to somewhat reniform, (17–)20–26 (–30) × (8–)10–12(–14)μ, contents golden yellow. Approximately one per cent of the spores are more or less globose and 12–15 microns in diameter, with or without thick walls. Zygospores and gemmae not observed. Multiplication by budding in liquid media.

Isolated from diseased roots of strawberry (*Fragaria* spp.), University Farm, St. Paul, Minnesota, July 1949. Description is based upon observations in diffuse daylight on potato-dextrose agar at 25–30 degrees C.

Type cultures deposited with the American Type culture collection, Washington, D. C., and Centraalbureau Voor Schimelcultures, Baarn, Netherlands.

On sterile potato slices in diffuse daylight the turf is 2–2.5 cm. in height, gray to ochre yellow; in bright white light, 200–400 f.c., 1–1.5 cm. in height, ochraceous orange. A dense turf similar in color to that on potato-dextrose agar is produced on Czapek solution in diffuse daylight, but growth is very sparse in darkness. During two years of growth on various laboratory media there has been a tendency for cultures to lose pigmentation.

Cultures were sent to Dr. G. A. Christenberry for examination and comparison with his "*Mucor* sp. no. 1" (Christenberry, 1940).[4] He stated that the author's isolates differed from "*Mucor* sp. no. 1" and that in his opinion the fungus could be designated as new.

Mucor recurvus differs from *Mucor oblongisporus* Naumoff in size, shape, and color of spores; size, shape, and color of columella, color of sporangia, and thickness of sporangiophores. It differs from *Mucor aromaticus* Povah in spore size, thickness of sporangiophores, shape and color of columella and odor of cultures.

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[4] Christenberry, G. A. 1940. A taxonomic study of the Mucorales in the Southeastern United States. *Jour. Elisha Mitchell Sci. Soc.* **56**: 333–366.

A NEW SPECIES OF CYSTOBASIDIUM FROM NEW JERSEY

LINDSAY S. OLIVE

(WITH 8 FIGURES)

During the Spring of 1951, the writer, with Dr. B. O. Dodge and Dr. D. P. Rogers of the New York Botanical Garden, made a foray into the New Jersey pine barrens for purposes of collecting fungi. During this excursion a number of collections of pine bark from dead standing trees of yellow pine were obtained. These pieces of bark all showed on their inner surface numerous insect tunnels which were densely spotted with small tan patches. These patches, upon microscopic examination, appeared to be the excreta of bark-boring insects and were composed primarily of fragments of fungus hyphae and bark. Within some of these patches there appeared an auriculariaceous fungus which has proved to be an undescribed species of *Cystobasidium* (FIGS. 1–8). Later and more numerous collections of pine bark obtained in the fall of 1951 from the same area failed to contain visible evidence of the fungus. A description of the new species follows:

Cystobasidium proliferans sp. nov.

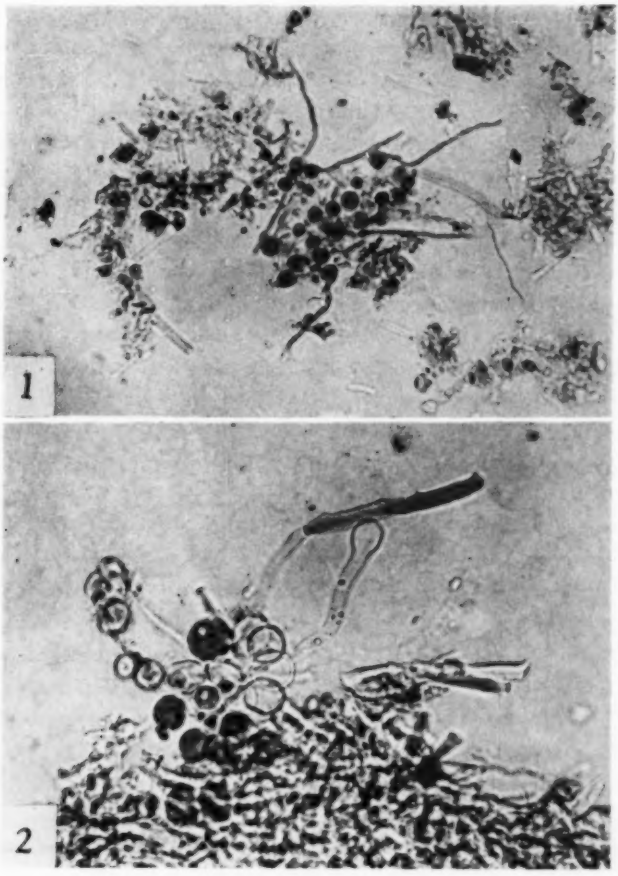
Fructificationes nullas distinctas efficiens. Hyphae 0.8–3.6(–5) μ diam.; probasidia globosa vel ample obovata, 10.5–11.5 × 11.9–16.8 μ, basidiis transverse triseptatis, 3.5–5.6 × 58.8–168 μ; basidiosporae allantoideae, 4–6.3 × 11.2–16.8 μ, frequenter demum uni- vel biseptatae.

No definite fructifications produced. Mycelial growth not abundant, hyphae septate, without clamp connections, 0.8–3.6(–5) μ diam.; probasidia typically arising in clusters of few to many, globose to broadly obovate, walls slightly thickened, measuring 10.5–11.5 × 11.9–16.8 μ, often proliferating within older, empty probasidia; basidia elongate, with 4-celled sporogenous portion and basal stalk-like cell of variable length, entire basidia measuring 3.5–5.6 × 58.8–168 μ; sterigmata awl-like; basidiospores allantoid,

apiculate, 4–6.3 × 11.2–16.8 μ, germinating by repetition or by germ tubes, frequently becoming 1–2-septate.

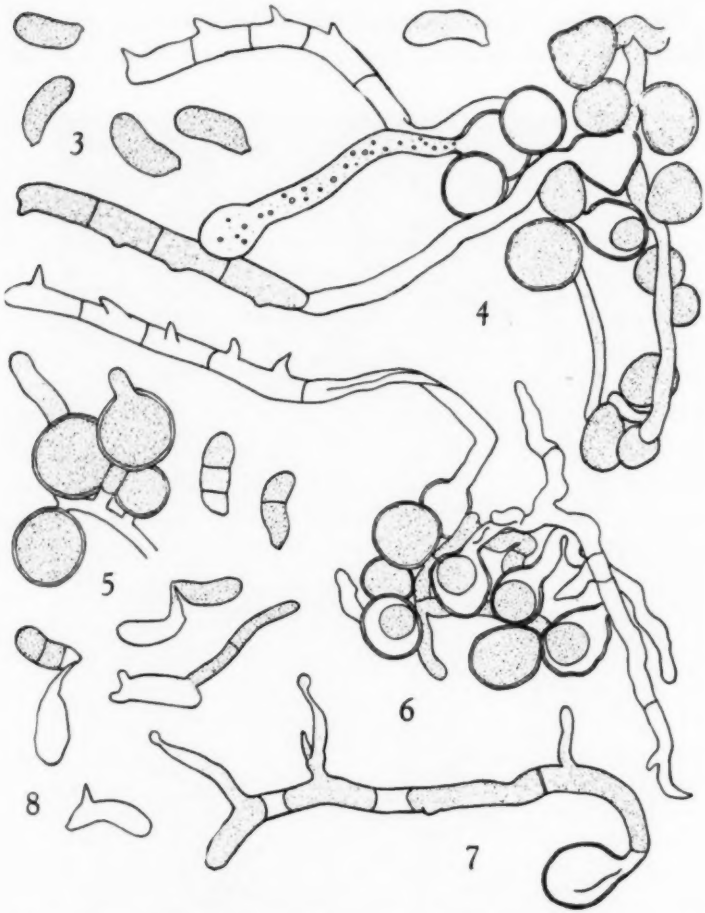
Growing on insect excreta on inner surface of bark of dead pines, near Lakehurst, New Jersey; May 5, 1951.

The basidiospore is characterized by having a truncate proximal end with a minute papillum where the spore was attached to the



FIGS. 1 and 2. Photomicrographs of *Cystobasidium proliferans*. 1. Group of probasidia, some of which are germinating, × 200. 2. Probasidia and basidia; note probasidial proliferation, × 450.

sterigma, very much as described by Couch (**1949**) in *Helicogloea alba* (Burt) Couch. Septate basidiospores were not observed in fresh material, but could be readily found in material kept in a



FIGS. 3–8. Camera lucida drawings of *Cystobasidium proliferans*. 3. Basidiospores. 4. Group of probasidia, some of which have produced basidia. Note probasidial proliferation. (Same as FIG. 2.) 5. Cluster of probasidia, two of which are germinating. 6. Probasidia, some of which show proliferation, and irregular basidia. 7. Empty probasidium with mature basidium. 8. Basidiospores showing septation and methods of germinating. All × 770.

moist chamber for several days (FIG. 8). Frequent abnormalities in basidial development were observed when the fungus was allowed to remain in the moist chamber for a few days. Some of the basidia developed more than the normal number of sterigmata, while others developed fewer or none and branched in a very irregular manner (FIG. 6).

A number of references have been made to the fact that the basidia of *Cystobasidium* are frequently bent abruptly at the point of juncture of the sporogenous portion and the stalk-like base. This appears to be a variable characteristic in the two previously described species and is generally not observed in the new species. Such a character is of very doubtful taxonomic value and should not receive undue emphasis.

The proliferation of probasidia is a striking feature of this fungus and resembles that described in *Platycarpa polypodii* Couch (**1949**). In the new species of *Cystobasidium*, as many as two successive proliferations have been observed within empty probasidia. As in previously described species of the genus, the probasidial wall is only slightly thickened. It is obviously not so thin as that of *Platygloea* or *Jola*, in which the probasidium tends to collapse when it is empty. Neither is it so thick as the wall of a typical teliospore of a rust, to which the probasidium of *Cystobasidium* has been compared.

In 1898, Lagerheim established *Cystobasidium* as a subgenus of *Jola* to accommodate his new species, *J. lasioboli*, which was found in Norway. Prior to this time three species of *Jola* had been described, all of which were parasites of mosses and all of which produced somewhat gelatinous or subgelatinous fructifications. *J. lasioboli*, however, was found to produce arachnoid growths over the surface of fructifications of *Lasiobolus equinus*. In addition, the probasidia were found to have somewhat thickened walls, in contrast to the thin-walled probasidia characteristic of *Jola*. Later, Gäumann (**1926**) recognized *Cystobasidium* as a genus with *C. lasioboli* as the only known species. No other species was added to the genus until 1939, when *C. sebaceum* Martin & Couch was described from Colombia. This fungus was found overgrowing remnants of an old *Sebacina* fructification on decaying wood. It

differs markedly from *C. lasioboli* in its production of thin gelatinous fructifications which are superficially like those of a *Sebacina*. The probasidia are clavate to spherical and have slightly thickened walls.

It is obvious that *C. proliferans* differs from either of the previously described two species in several important respects. It differs from both in lacking a visible fructification and in the proliferation of its probasidia. It further differs from *C. lasioboli* in the shape and size of its probasidia and basidiospores and from *C. sebaceum* in its microdimensions. The new species apparently represents the first record of a *Cystobasidium* from North America.

The status of Rick's new species, *Jola orthosacca*, from Brazil (**1933**) is not clear. The fungus was found growing on wood in effuse, somewhat gelatinous patches. The probasidia are described as oval-cylindric, and the basidiospores are cylindric and measure 5×8–$10\,\mu$. It is doubtful that such an effuse saprophyte on dead wood should be placed in a genus such as *Jola*, which is commonly recognized as having small restricted fructifications that parasitize mosses. Neither does it fit well into the genus *Cystobasidium*, although it would seem more advisable to include it in this genus as a possible transitional form rather than to allow it to remain in *Jola*. The writer has not yet succeeded in obtaining a specimen of Rick's fungus for examination and is therefore not in a position to make a definite change in the taxonomic status of the fungus at this time.

For interesting discussions of relationships among the probasidium-forming Auriculariaceae and the possible relationships between this and other groups of heterobasidiomycetes, the reader is referred to the papers of Martin (**1945**) and Couch (**1949**).

The writer is grateful to Dr. D. P. Rogers for preparing the Latin description. Type material of the new species has been deposited in the mycological collections at the New York Botanical Garden.

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Couch, J. N. The taxonomy of *Septobasidium polypodii* and *S. album*. Mycologia **41**: 427–441. 1949.

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NOMENCLATURAL NOTES ON THE ERYSIPHACEAE[1, 2]

WM. BRIDGE COOKE[3]

Since the publication of Salmon's monograph on the Erysiphaceae (Mem. Torr. Bot. Club **9**: 1–292. 1900), subsequent authors for the most part have accepted his nomenclature. Unfortunately, Salmon followed the American Code of Botanical Nomenclature and thus the nomenclature of this family is based largely on work published between 1800 and 1820.

At the Stockholm meetings of the International Botanical Congress held in the summer of 1950 the starting points of the nomenclature of the fungi were fixed by month, date, and year. While the corrected rules have not yet been published, the Stevenson and Rogers report (Plant Disease Reporter **34**: 327–333. 1950) of the changes made can be used as a reference. Here the starting point for these fungi, included in the Fungi caeteri (Art. 20, par. f.), is stated as: (f) Fungi caeteri, 1821 (Fries, *Systema mycologicum*, vol. 1). "Vol. I of the *Systema* is treated as having appeared Jan. 1, 1821, and the *Elenchus fungorum* (1828) is considered to be part of the *Systema*. Names of *Fungi caeteri* published in other works between the dates of the first and last parts of the *Systema* which are synonyms or homonyms of names of any of the *Fungi caeteri* included in the *Systema* do not affect the nomenclatorial status of names used by Fries in this work."

It thus becomes necessary to review the names in common use in this family and to replace some of them that are not now in conformity with the rules. The situation has not previously been corrected, possibly for one or more of several reasons. The Salmon monograph was considered satisfactory; because it was satisfactory

[1] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

[2] Scientific Paper No. 1070, Washington Agricultural Experiment Stations, Pullman. Project No. 1047.

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no one needed to take time from more important current research to correct it. Workers with the group felt no need to conform with any special set of rules as long as a name was available for the organism with which they were working. Until the Stockholm meetings there was no clear-cut definition of the starting point of "Fungi caeteri." One either started with each individual volume of Fries; or one started with Vol. I of Fries assuming any date in 1821 depending on whether or not one wanted to use S. F. Gray; or one relied on earlier work without regard to starting points. The definition of the publication dates of Fries's volumes now makes it possible to bring the names and author-citations used by Salmon "up-to-date" so to speak, that is, to bring them in line with the latest version of the International Rules of Botanical Nomenclature, with considerable facility. In doing this it should be remembered we need be concerned only with those names in use prior to 1832, and especially those in use between 1821 and 1832 which are common to the modern literature of the powdery mildews.

The first person after Jan. 1, 1821, to discuss this group was Mérat (Nouvelle Flore des environs de Paris, 2nd ed., vol. 1, Paris. 1821) who listed a number of species of *Erysiphe* just prior to June 15, 1821 (Rogers, Mycologia **43**: 377. 1951). Later in that year, S. F. Gray (Natural Arrangement of British Plants, 1821) listed a number of species of *Erysibe*. Fries's own work on the group did not appear until 1829 in the first part of the third volume of the *Systema*. Here he treated most of the species under *Erysiphe*.

According to the revised rules, those genera Fries used must continue to be used although if another author between 1821 and 1832 proposed other genera they may also be used if they do not interfere with the use of Fries's genera. According to the Rules, if Fries used a specific name in the *Systema* or the *Elenchus* between 1821 and 1832 that name must stand regardless of other names applied to the organism by other workers.

The following, then, become the corrected forms of the names applicable to species of erysiphaceous fungi known prior to 1832. The first name listed is in conformity with the rules, the second name is that which was used by Salmon, and other workers follow-

ing him, if incorrect. Other names necessary to clear up synonymy are cited. To retain Salmon's concepts of varieties it has been necessary to make new varietal combinations under the specific names found to be correct according to the present wording of Article 20. The complete citation for the name Salmon used is given. Complete synonymy according to Salmon may be found in his monograph.

Podosphaera clandestina (Wallr. ex Fries) Lév. Ann. Sci. Nat. Bot. III. **15**: 36. 1851.

Podosphaera oxyacanthae (DC.) de Bary, Beitr. Morph. Phys. Pilze **1** (XIII): 48. 1870.

Erysiphe clandestina Wallr. ex Fries, Syst. Myc. **3**: 238. 1829.

Podosphaera clandestina var. **tridactyla** (Wallr.) comb. n.

Podosphaera oxyacanthae var. *tridactyla* (Wallr.) Salmon, Mem. Torr. Bot. Club **9**: 36. 1900.

Sphaerotheca **macularis** (Wallr. ex Fries) comb. n.

Sphaerotheca humuli (DC.) Burr. Bull. Ill. State Lab. Nat. Hist. **2**: 400. 1887.

Erysiphe macularis Wallr. ex Fries, Syst. Myc. **3**: 237. 1829.

Sphaerotheca macularis var. **fuliginea** (Schlecht. ex Fries) comb. n.

Sphaerotheca humuli var. *fuliginea* (Schlecht.) Salmon, Mem. Torr. Bot Club **9**: 49. 1900.

Sphaerotheca pannosa (Wallr. ex Fries) Lév. Ann. Sci. Nat. Bot. III. **15**: 138. 1851.

Sphaerotheca pannosa (Wallr.) Lév.

Erisiphe pannosa Wallr. ex Fries, Syst. Myc. **3**: 236. 1829.

Uncinula salicis (DC. ex Mérat) Wint. in Rab. Krypt. Fl. Deutschl. **1** (2): 40. 1884.

Uncinula salicis (DC.) Wint.

Erysiphe salicis DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 131. 1821.

Uncinula bicornis (Fr.) Lév. Ann. Sci. Nat. Bot. III. **15**: 153. 1851.

Uncinula aceris (DC.) Sacc. Syll. Fung. **1**: 8. 1882.

Erysiphe bicornis Fries, Syst. Myc. **3**: 244. 1829.

Uncinula bicornis var. **tulasnei** (Fckl.) comb. n.

Uncinula aceris var. *tulasnei* (Fckl.) Salmon, Mem. Torr. Bot. Club **9**: 93. 1900.

Uncinula prunastri (DC. ex Mérat) Sacc. Syll. Fung. **1**: 7. 1882.
Uncinula prunastri (DC.) Sacc.
Erysiphe prunastri DC. ex Mérat, Nouv. Fl. ed 2. **1**: 134. 1821.

Uncinula **adunca** (Fr.) comb. n.
Uncinula clandestina (Biv. Bern.) Schroet. in Cohn, Krypt. Fl. Schles. **3**: 245. 1893.
Erysiphe adunca Fries, Syst. Myc. **3**: 245. 1829.

Microsphaera berberidis (DC. ex Mérat) Lév. Ann. Sci. Nat. Bot. III. **15**: 159. 1851.
Microsphaera berberidis (DC.) Lév.
Erysiphe berberidis DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 133. 1821.

Microsphaera euonymi (DC. ex Mérat) Sacc. Syll. Fung. **1**: 11. 1882.
Microsphaera euonymi (DC.) Sacc.
Erysiphe euonymi DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 132. 1821.

Microsphaera astragali (DC. ex Mérat) Trev. Spighe e Paglie **1**: 39. 1853.
Microsphaera astragali (DC.) Trev.
Erysiphe astragali DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 132. 1821.

Microsphaera penicillata (Wallr. ex Fries) Lév. Ann. Sci. Nat. Bot. III. **15**: 153. 1851.
Microsphaera alni (Wallr.) Salmon, Mem. Torr. Bot. Club **9**: 129. 1900.
Erysiphe penicillata Wallr. ex Fries, Syst. Myc. **3**: 243. 1829.

Microsphaera penicillata var. **lonicerae** (Fr.) comb. n.
Microsphaera alni var. *lonicerae* (DC.) Salmon, Mem. Torr. Bot. Club **9**: 142. 1900.

Microsphaera penicillata var. **rhamni-frangulae** (Fr.) comb. n.
Microsphaera alni var. *divaricata* (Wallr.) Salmon, Mem. Torr. Bot. Club **9**: 146. 1900.
Erysiphe penicillata var. *rhamni-frangulae* Fries, Syst. Myc. **3**: 244. 1829.

Microsphaera penicillata var. **vaccinii** (Schw.) comb. n.
Microsphaera alni var. *vaccinii* (Schw.) Salmon, Mem. Torr. Bot. Club **9**: 140. 1900.

Microsphaera penicillata var. **extensa** (Cke. & Pk.) comb. n.
Microsphaera alni var. *extensa* (Cke. & Pk.) Salmon, Mem. Torr. Bot. Club **9**: 152. 1900.

Microsphaera penicillata var. **calocladophora** (Atk.) comb. n.
Microsphaera alni var. *calocladophora* (Atk.) Salmon, Mem. Torr. Bot. Club **9**: 153. 1900.

Microsphaera penicillata var. **ludens** (Salmon) comb. n.
Microsphaera alni var. *ludens* Salmon, Mem. Torr. Bot. Club **9**: 154. 1900.

Microsphaera grossulariae [Wallr.] Lév. Ann. Sci. Nat. Bot. III. **15**: 160. 1851.
Microsphaera grossulariae (Wallr.) Lév.

Erysiphe polygoni DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 132. 1821.
Erysiphe polygoni DC. Fl. Fr. **2**: 273. 1805.

Erysiphe communis Wallr. ex Fries, Syst. Myc. **3**: 239. 1829.
Erysiphe cichoracearum DC. Fl. Fr. **2**: 274. 1805.

Erysiphe galeopsidis DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 134. 1821.
Erysiphe galeopsidis DC. Fl. Fr. **6**: 108. 1815.

Erysiphe graminis DC. ex Mérat, Nouv. Fl. ed. 2. **1**: 133. 1821.
Erysiphe graminis DC. Fl. Fr. **6**: 106. 1815.

Phyllactinia guttata (Fries) Lév. Ann. Sci. Nat. Bot. III. **15**: 144. 1851.
Phyllactinia corylea (Pers.) Karst. Act. Soc. Faun. Fl. Fenn. **2**: 92. 1885.
Erysiphe guttata Fries, Syst. Myc. **3**: 245. 1829.

The writer wishes to take this opportunity to apologize to the late O. Kuntze and N. J. McGinty for borrowing their methods of creating new combinations. However, names were needed which would be in conformity with the rules and several years would be required to monograph this family in the light of such recent works as that by S. Blumer (Die Erysiphaceen Mitteleuropas, Beiträge zur Kryptogamenflora der Schweiz **7** (1): 1–483. 1933) and the masses of material available for study in mycological herbaria in North America as well as elsewhere.

The writer wishes to take this opportunity to thank J. A. Stevenson for reading the preliminary manuscript, and C. G. Shaw for reading the manuscript and offering criticisms.

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A NEPHELOMETRIC TECHNIQUE FOR LARGE CULTURES

GEORGE H. SCHERR

(WITH 3 FIGURES)

It is sometimes necessary to determine growth curves nephelometrically on larger cultures of organisms than can be contained by the cells of the nephelometers. One solution is to transfer suitable aliquots of the culture to these cells. Unless sterile technique is maintained throughout this operation there is a loss in culture

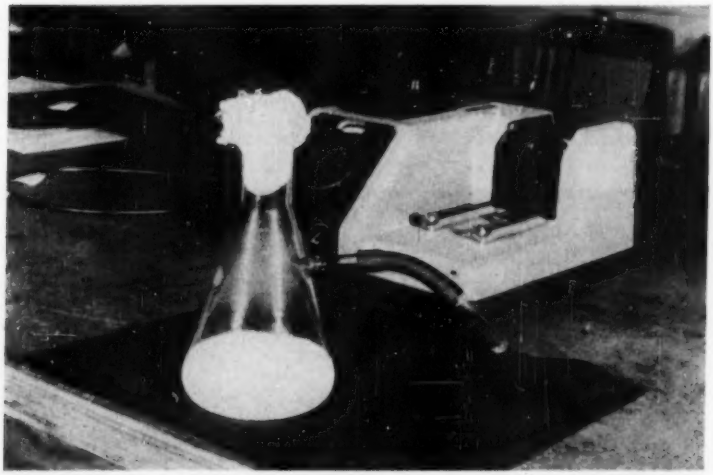

FIG. 1. The culture flask with the cell from the nephelometer attached to the side arm.

material. In addition, repeated nephelometric determinations increase the chance of contamination of the culture. The following unit was devised to overcome these difficulties.

A small side-arm is affixed to an Erlenmeyer flask and the cell to be used in the nephelometer is attached to the side-arm through

FIG. 2. The culture flask tilted to fill the cell prior to taking a reading.

a piece of rubber tubing. Suitably stoppered and filled with medium, the entire unit may be autoclaved (FIG. 1). After inoculation and incubation the culture is shaken and the cell filled by tilt-

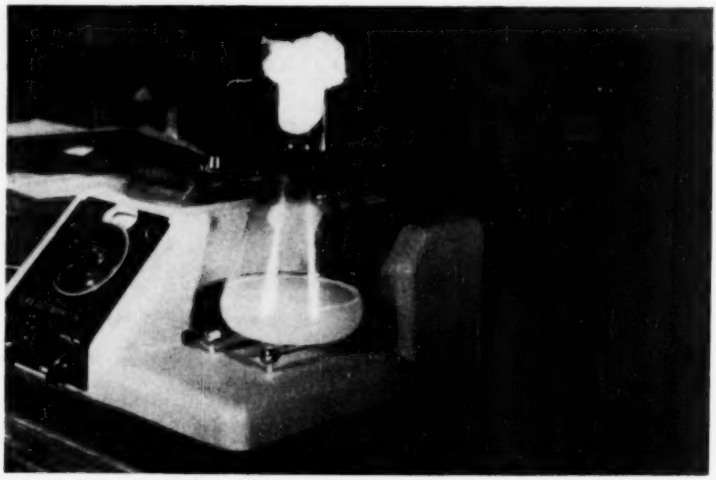

FIG. 3. The cell containing the culture inserted in the chamber of the nephelometer.

ing the flask (FIG. 2). The cell is inserted in the nephelometer and the reading taken against a standard. The cell is then emptied by tilting and the culture is returned to the incubator.

In this particular case a Fisher Electrophotometer is being used as a nephelometer, necessitating readings in a darkened room, since the lid for the cell enclosure cannot be closed. Other nephelometers would not present this problem.

I have found this technique particularly useful in working with yeast cultures which, under certain conditions, tend to form filaments and rosettes of cells, thus making the plate-counting technique impractical.

ACKNOWLEDGMENT

The author is indebted to Miss Pauline R. Kelly, Medical Photographer at the Creighton University School of Medicine, for the photographs.

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NOTES AND BRIEF ARTICLES

A Refrigerator Cabinet for Fungal Cultures

In 1947 the writers had constructed in the shops of the Philadelphia Quartermaster Depot for use in the Biological Laboratories of that installation a stainless steel cabinet for holding test tube slants of fungous cultures, and more recently two cabinets of similar design were constructed by a firm in Boston for use at the Farlow Herbarium of Harvard University. The three cabinets differ only in minor detail. They were made to fit ordinary household refrigerators. They have proven to be satisfactory and so convenient and saving of time and space over the wire basket method that it is felt that a note on the specifications might be useful to others. The idea is not new. Cabinets of a more or less similar nature have been seen in several laboratories. Those illustrated in this note do perhaps represent something of a refinement over some of those previously seen. Raper, Thom, and Fennel (A Manual of the Penicillia, p. 80. 1949) show a view of cabinets of somewhat similar design in which the trays are fashioned of perforated brass sheeting to allow maximum air circulation.

A general view of the cabinet and refrigerator in use at Philadelphia is shown in Fig. 1 (photo by Photographic Department, Philadelphia Quartermaster Depot).

In Fig. 2 are presented diagrams and specifications of one of the cabinets at Harvard. The overall dimensions may of course be modified to suit the particular refrigerator at hand. That in Fig. 2 was designed for a Hotpoint, model EB 8–2 or EB 8–4, size 8 cubic feet, having an inside height of 41½ in., a width of 23 in., and a front to back depth of 15 in. Each tray holds 2 rows of 16 × 150 mm. test tubes making 60 test tubes per tray and a total capacity of 1260 cultures for the cabinet. Using 13 × 100 mm. tubes, which is also a satisfactory size, 3 rows may be placed in each tray, 102 per tray, or a total of 2142. This cabinet was made in 1949 by the Wrought Iron Kitchen Equipment Company,

Boston, Mass., using polished stainless steel, finished in a first-class manner with no rough edges, for $186.00. It is important that the steel be of a proper gauge to avoid excess weight and yet provide ample rigidity. A length of glass tubing of suitable thickness

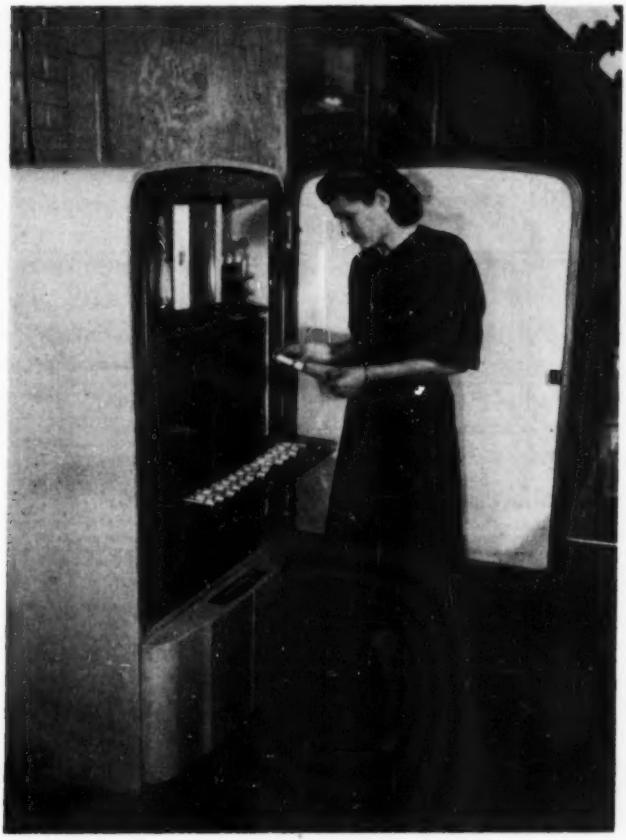

FIG. 1.

placed between the rows of tubes prevents any lengthwise slippage and each culture tube is then held firmly in place. In practice there has been no apparent reason for having the bottoms of the trays perforated.

Where cultures are to be kept in the growing condition, rather than in the lyophilized state, the tray method has several desirable features. The trays are readily carried to and from the transfer room. The cultures are always in order and easily located. The label or crayon marks are on the upper side of the tubes where they may be read at a glance and where they do not rub off. The tubes are placed flat on the tray with the agar surface up, thus making the colony easily observed. Large numbers of slants may be

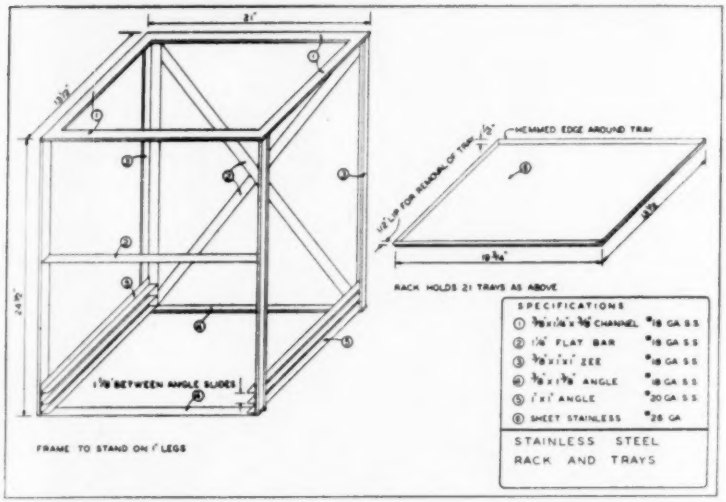
HEMMED EDGE AROUND TRAY
RACK HOLDS 21 TRAYS AS ABOVE
SPECIFICATIONS
① CHANNEL #18 GA S.S.
② FLAT BAR #18 GA S.S.
③ ZEE #18 GA S.S.
④ ANGLE #18 GA S.S.
⑤ ANGLE #20 GA S.S.
⑥ SHEET STAINLESS #26 GA
STAINLESS STEEL RACK AND TRAYS
BETWEEN ANGLE SLIDES
FRAME TO STAND ON 4' LEGS

FIG. 2.

quickly scanned for evidence of contamination without the necessity of handling the tubes individually. The collection so kept is compact, neat, clean, and attractive.—W. LAWRENCE WHITE AND R. T. DARBY.

FEMSJONIA LUTEOALBA

In my recent revision of the North Central Tremellales (Univ. Iowa Stud. Nat. Hist. **19** (3): 36. 1952), I proposed the new combination *Femsjonia radiculata* (Fries) Martin for the species widely known as *F. luteoalba* Fries. This was based on Fries's

statement, when he established the genus *Femsjonia* (Summa Veg. Scand. 348. 1849), that the species was the same as *Peziza radiculata* Sowerby (Engl. Fungi *Pl. 114.* 1797), accepted by Fries and listed in Syst. Myc. **2**: 81. 1822. I am indebted to Dr. R. P. Korf for calling my attention to the fact that Nannfeldt (Svensk Bot. Tidskr. **32**: 118. 1938) based the discomycetous genus *Sowerbyella* on *Peziza radiculata* Sow. ex Fries. Nannfeldt examined Sowerby's type, now at Kew, and reports that Miss Wakefield had previously examined it and had annotated the sheet. There can be no question, therefore, that the original of Sowerby's *Pl. 114* is a discomycete. The combination *Femsjonia radiculata* is in consequence invalid and the earlier synonyms cited under that name in the Revision: *Macroscyphus radiculatus* S. F. Gray, *M. Sowerbea,* S. F. Gray, *Peziza Sowerbea* Pers. and *Peziza radiculata* Sow. ex Fries are to be regarded as names applied to the discomycetous species *Sowerbyella radiculata* (Sow. ex Fries) Nannf. Nannfelt does not list Gray's names, but at the time he published these were regarded as pre-Friesian.

The next name cited is *Exidia pezizaeformis* Lév. (Ann. Sci. Nat. Bot. III. **9**: 127. 1848. Léveillé's description applies fairly well to *Femsjonia luteoalba,* but he cites two collections, one on oak, the other on pine, the latter a most improbable substratum for *Femsjonia.* Fries, in a later publication (Hymen. Eur. 696. 1874), refers to Léveillé's species in connection with *Femsjonia,* with the comment: "Utrum synonyma sit, an altera hujus generis species, non liquet." Nevertheless, Karsten (Bidr. Finl. Nat. Folk **25**: 352. 1876) made the combination *Femsjonia pezizaeformis* (Lév.) Karst. for what is clearly Fries's species. His description is evidently based on specimens collected in Finland and there is no indication that he had seen authentic material of Léveillé's species. The only specimen of *Exidia pezizaeformis* I have seen is one at Kew, collected and determined by Léveillé himself, but not, apparently, the type. I did not examine it microscopically, but it is certainly not a *Femsjonia.* It is probably an *Exidia* or possibly an *Auricularia.*

In any event, *Femsjonia radiculata* is an unwarranted and useless name which will merely encumber synonymy. The well-established

name *Femsjonia luteoalba* Fries should designate the species concerned until there is convincing evidence that there is an earlier valid specific epithet.—G. W. MARTIN.

UNDERWOODIA IN KANSAS

Kansas can now be added to the list of states having in its fungous flora the rare discomycete, *Underwoodia columnaris* Peck. On June 6, 1951, this fungus was found in moist humus on a steep wooded slope of a small side ravine of Hackberry Glen, south of Manhattan, Riley County, Kansas. The dominant trees in this locality were *Ostrya virginiana* and *Quercus muhlenbergii*. Five specimens of *Underwoodia* were collected at this time and later, on June 9, three additional specimens were found a short distance away on the east-facing slope of the main ravine.

The largest specimen was eighteen centimeters high and nine centimeters broad at the base, tapering upwards. Some of the specimens were fused at the base, thus appearing double. It is interesting to note that the occurrence of *Underwoodia columnaris* in Kansas coincides with a period of excessive rainfall, more than ten inches of rain having fallen in May and the earlier part of June.

The Kansas collections extend the known distribution of this fungus both west and south. It has been recorded previously from New York, Michigan, Illinois, Manitoba, and Iowa.

The specimens have been deposited in the herbaria of Kansas State College, Cornell University, and the United States Department of Agriculture at Beltsville, Maryland.—CLARK T. ROGERSON AND ROBERT L. SHAFFER.

WANTED

SEAVER, NORTH AMERICAN CUP-FUNGI (OPERCULATES) 1928. Any persons having copies they wish to dispose of, please communicate with the author, 201 Alexander Place, Winter Park, Florida.

REVIEWS

RESEARCHES ON FUNGI. VOL. 7. THE SEXUAL PROCESS IN THE UREDINALES, by A. H. Reginald Buller. xx + 458 pp. Portrait and 124 figs. Published for the Royal Society of Canada by the University of Toronto Press. 1950. Price, $13.50.

This posthumous volume by the late Dr. A. H. Reginald Buller is without doubt the most important contribution to uredinology that has appeared in at least two decades.

The book is divided into twelve chapters. The first five deal primarily with an historical account of pycnia (pycnidia), including comparison of similar structures in lichens and Ascomycetes, and the discovery of their sexual function.

Chapters 6 and 7 deal with the sexual processes in rusts and a comparison of these processes with those of other Basidiomycetes. Chapter 8 reviews cytological work as related to sexuality in heterothallic species, chapter 9 discusses the work done on *Cronartium ribicola*, chapter 10 with that on *Puccinia suaveolens* and chapter 11 with that on *Gymnosporangium*. A final chapter discusses evolutionary history of the rusts with consideration of the evolution of other entomophilous fungi.

In chapters 3 and 4 are included for the first time the details of Dr. Buller's own observations on flexuous hyphae and their function in the sexual process. And in chapter 10 is to be found the only detailed account as yet available of his extensive studies on the thistle rust.

Not all mycologists or uredinologists will agree with Dr. Buller's denial of a "male" function of the pycniospores, and those, including the present reviewer, who saw the results of the inoculations while growing, will agree that Dr. Buller was not justified in explaining away, on grounds of faulty technique, the report of Dr. D. L. Bailey that in the strain he was using *Puccinia helianthi* occasionally short-cycled to the brachy-type of life cycle.

In his discussion of the unique autoecious *Gymnosporangium bermudianum* Buller suggests the possibility that the appearance

of aecia on the galls of the juniper host, which later produce telia, may be due to de-diploidization of a binucleate mycelium derived from aeciospore infection. This seems a logical possibility and may prove to be the explanation for the origin of that species as well as of a series of species which show evidence of correlation with long-cycled heteroecious species but which apparently carry out their full life cycle on the host which would normally be the host for the diplout.

Repeated use of the expression "opposite sex" as applied to haploid mycelium or pycniospores, while short and convenient and reflecting Dr. Buller's concept of sexuality in the rusts and other Basidiomycetes, will not be acceptable to those who think of the long-cycled, heterothallic rusts as fundamentally (phylogenetically?) hermaphroditic, self-sterile and cross-fertile.

As a whole, the volume is a thorough and stimulating historical review of the development of modern ideas of the Uredinales, with the addition of much that is new. It should be in every mycological and phytopathological library.

Previous volumes of the series were published privately by Dr. Buller and printed by Longman's in London, England. The present volume, sponsored by the Royal Society of Canada, was printed by the University of Toronto Press. The format follows closely that of the former volumes, the paper is of even better grade.—H. S. JACKSON.

[Few readers of MYCOLOGIA will not have seen Volume 7 of Buller's Researches by now. The review of this important book was entrusted to Dr. Jackson, but his final illness and death prevented him from submitting it. Professor Bailey and Miss Margaret Moffat, his secretary, sent his notes, which are reproduced above, with very slight modification, in the belief that many readers of the journal will be interested in Dr. Jackson's comments. —ED.]

TAXONOMY OF THE PYRENOMYCETES, by E. S. Luttrell. University of Missouri Studies, Vol. 24, No. 3. 120 pp. 1951. Price $2.50.

The aims of this study are, as stated in the introduction, (1) to review the chief suggestions as to changes in the system of classification of the Pyrenomycetes which have been advanced since the

publication of Lindau's system, (2) to examine critically the data on the structure of the ascus and the ascocarp centrum in this group, and (3) to present a revision of the Pyrenomycetes as a basis for the discussion of these proposals and as a means of evaluating the taxonomic significance of variations in ascus and centrum structure.

Asci are divided primarily into those with a double wall, the bitunicate type, and those with a single wall, the unitunicate type, and typical instances of both types are discussed. The term centrum is adopted, replacing the older, ambiguous "nucleus," to denote the ascogenous hyphae and asci and the associated sterile structures occupying the cavity in which all develop. On the basis of variation in centrum structure, eight developmental types are recognized in the Pyrenomycetes, each of which is described in some detail. As a result of the considerations advanced, a revised classification of the Ascomycetes is presented. It is admittedly provisional, but the author believes it is in approximate accord with the known facts.

The author has performed a valuable service in bringing together scattered references on this difficult problem and in subjecting the data to careful analysis. The work deserves careful study by all who are interested in a modern approach to this large and important group of fungi.—G. W. MARTIN.

MANUSCRIPT

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